資料2

# 感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

## １ 基本的な方針

運営委員会に報告する資料においては、

(1) 文献報告は、同一報告に由来するものの重複を廃した一覧表を作成すること。

(2) 8月の運営委員会において、国内の輸血及び血漿分画製剤の使用した個別症例の感染症発生報告は、定期的にまとめた「感染症報告事例のまとめ」を運営委員会に提出する取り扱いとされた。これにより、感染症定期報告に添付される過去の感染症発生症例報告よりも、直近の「感染症報告事例のまとめ」を主として利用することとすること。

## ２ 具体的な方法

(1) 感染症定期報告の内容は、原則、すべて運営委員会委員に送付することとするが、次の資料概要を作成し、委員の資料の確認を効率的かつ効果的に行うことができるようにする。

① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第2）及び該当文献を添付した**「資料概要A」**を事務局が作成し、送付する。

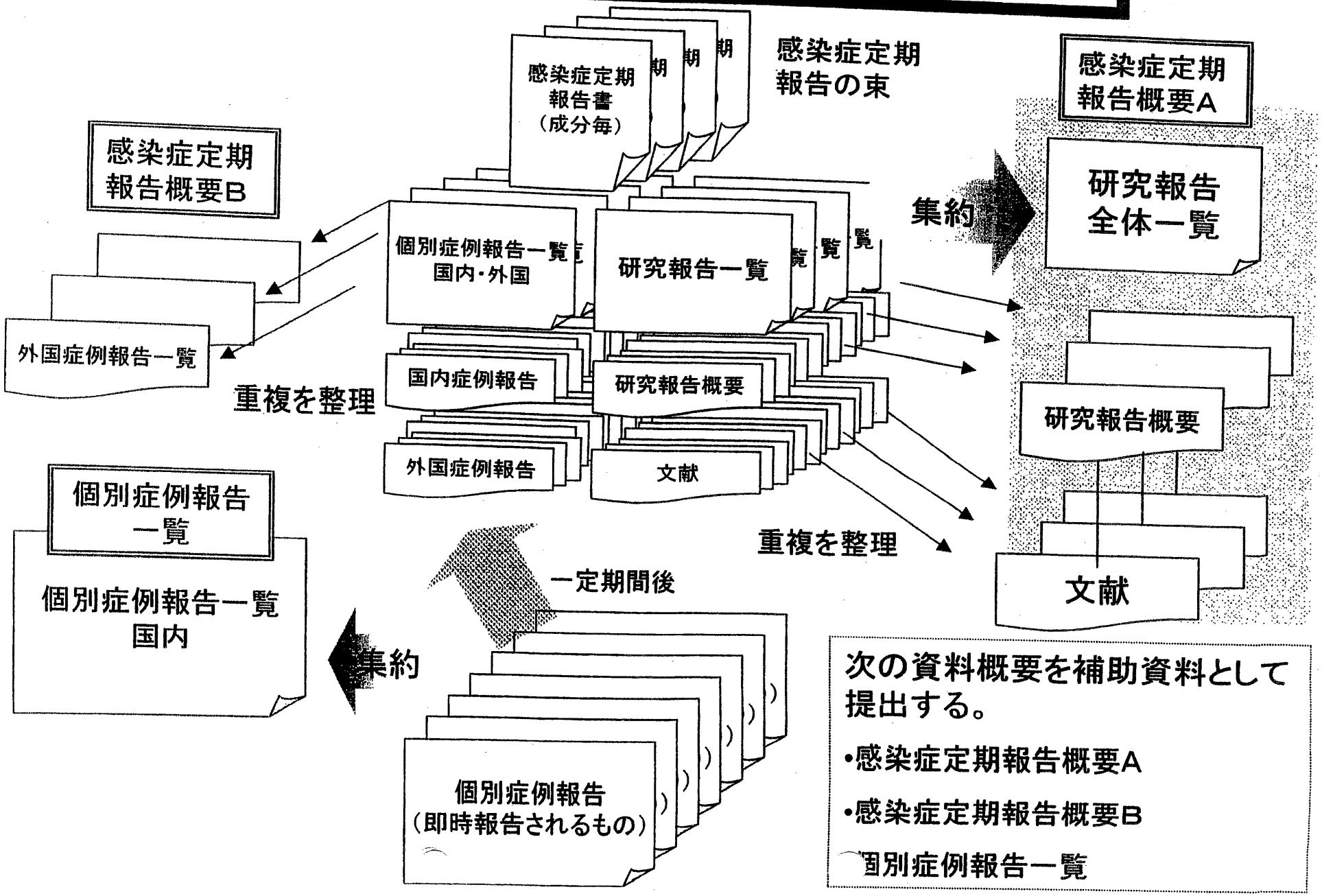
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

③ 感染症発生症例報告のうち、発現国が「国内」の輸血による症例及び血漿分画製剤の使用による感染症症例については、「感染症報告事例のまとめ」を提出することから、当該症例にかかる「資料概要」は作成しないこととする。ただし、運営委員会委員から特段の議論が必要との指摘がなされたものについては、別途事務局が資料を作成する。

(2) 発現国が「外国」の感染症発生症例報告については、国内で使用しているロットと関係がないもの、使用時期が相当程度古いもの、因果関係についての詳細情報の入手が困難であるものが多く、必ずしも緊急性が高くないと考えられるものも少なくない。また、国内症例に比べて個別症例を分析・評価することが難しいものが多いため、緊急性があると考えられるものを除き、その安全対策への利用については、引き続き、検討を行う。

(3) 資料概要A及びBについては、平成16年9月の運営委員会から試験的に作成し、以後「感染症的報告について（目次）」資料は廃止することとする。

# 感染症定期報告・感染症個別症例報告の取り扱い




次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧

# 感染症定期報告概要

（平成２０年５月２１日）

平成１９年１２月１日受理分以降

Ａ　研究報告概要
Ｂ　個別症例報告概要

# Ａ　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

１　平成１９年１２月１日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

２　一覧表においては、前回の運営委員会において報告したもの以降の研究報告について、一覧表の後に当該感染症の主要研究報告の内容を添付した。

# 感染症定期報告の報告状況(2007/12/1～2008/2/29)

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | 新出文献No. |
|---|---|---|---|---|---|---|
| 70171 | 2007/12/25 | 70823 | A型肝炎 | 第55回日本ウイルス学会学術集会 2P213 | 遺伝子型の異なる複数のHAV細胞馴化株における加熱や加圧による不活化効果を検討した。25%アルブミン存在下60℃10時間加熱処理または室温下300～420MPaの1分間加圧3サイクルに対し、HAV細胞馴化株間で不活化効果に差が見られた。Validation試験に使用する株として、加熱や加圧で不活化されにくく細胞で良く増殖するKRM238が適切と考えられた。血液製剤の製造工程に新規不活化法を導入する場合にはValidation試験に使用する株を適切に選定する必要がある。 | 1 |
| 70168 | 2007/12/20 | 70810 | B型肝炎 | Transfusion 2007; 47: 1162-1171 | 日本赤十字のスクリーニングシステムでHBsAg及び抗B型肝炎コア抗原抗体が陰性であったHBV DNA陽性供血者26名において急性HBV感染におけるウイルスマーカーの動態を調べた。検出可能期間の中央値は、HBV DNAが個別NATで74日、MP NATで50日、HBsAgが42日であった。26名中6名は変異型ウイルスに感染し、うち3名ではHBsAgが検出できなかった。HBV NATは、MPで行ったとしても、HBsAg検査よりも効果的で、HBsAgウインドウ期前後の感染供血者を排除することができる。 | |
| 70168 | 2007/12/20 | 70810 | B型肝炎 | Transfusion 2007; 47: 1197-1205 | 日本赤十字血液センターに保管されている1997-2004年の反復供血者の全供血の遡及調査を行い、ID-NATのみHBV陽性である血液由来の血液製剤の輸血によるHBV伝播リスクを検討した。HBV ID-NATを実施したHBV転換供血者の保管血液15,721本中158検体 (1.01%) が陽性であった。スクリーニングをすり抜けたHBc 抗体価の低いオカルトHBVキャリア由来の血液製剤を原因とするHBV感染リスクは、HBsAg発現前やMP-NATウインドウ期の供血による伝播リスクよりも10倍以上低い。 | |
| 70168 | 2007/12/20 | 70810 | B型肝炎 | 第31回日本血液事業学会総会 2007年10月 3-5日 | 平成19年3月、輸血によるHBV感染が疑われるとの報告が千葉県赤十字血液センターにあった。因果関係の確認のために実施した当該輸血用血液製剤に係る保管検体個別NATは陰性であり、献血者追跡調査を行った。1名の献血者が平成19年1月にB型肝炎を発症したとの情報が得られ、調べたところ、献血者のHBV-DNAは患者のそれと塩基配列が一致した。20プールNAT陰性、HBV保管検体個別NAT陰性であったが、献血者追跡調査により輸血用血液製剤からのHBV感染が示唆された症例であった。 | |
| 70168 | 2007/12/20 | 70810 | B型肝炎 | 第31回日本血液事業学会総会 2007年10月 3-5日 一般演題51 | 2004年8月よりNATスクリーニングのプールサイズを50から20に縮小した。大阪府赤十字血液センターで検出されたHBV-NAT陽性事例81人を基にプールサイズ縮小の効果等について解析を行った。プールサイズ縮小後に100コピー未満/mLのHBV-NAT陽性者の比率が高くなっていることから、縮小による効果があると思われた。追跡調査、遡及調査及び医師の面談等による総合的な解析によりHBV低濃度キャリアが疑われる献血者がプールサイズ縮小後に多く検出されていることが推察された。 | |
| 80008 | 2008/01/25 | 70856 | C型肝炎 | Clin Vaccine Immunol published online doi:10.1128 | 抗HCV抗体陰性で、肝組織中のHCV RNA検出により潜在性HCV感染と診断された110例の患者由来の血清中のGOR抗体反応性を調べた。抗GOR IgG陽性患者は22例(20%)で、慢性C型肝炎患者での陽性率(70/110、63.6%)に比べ有意に低かった。HCVに無関係の肝疾患患者120例では抗GOR IgGは全く検出されなかった。市販の検査でHCV特異抗体を検出できず、血清中HCV RNAが検出できない患者で抗GOR IgG検査を行う事は、肝生検なしで潜在性HCV感染を同定する手助けとなりうる。 | 2 |
| 70168 | 2007/12/20 | 70810 | E型肝炎 | Arch Virol 2007; 152: 1623-1635 | 日本においてHEVの不顕性感染が増加しているかを調べるため、1991-2006年の献血者のうちHEV感染の可能性のあるALT 61IU/L以上の4019名から得られた血清検体中の抗HEV IgG、抗HEV IgMおよびHEV RNAを調べたところ、2004-2006年の献血者のHEV陽性率は1998年のそれと同等であった。またALT 201IU/L以上の献血者についても1991-1995年、1996-1999年および2004-2006年でHEV陽性率の差は見られなかった。 | 3 |
| 70168 | 2007/12/20 | 70810 | E型肝炎 | J Med Virol 2007; 79: 734-742 | 日本におけるアラニンアミノトランスフェラーゼ(ALT)高値供血者の無症候性E型肝炎感染の現況を調べた。日本赤十字血液センターでALT高値(61-476 IU/L)の献血者6700名の血清検体を検査したところ、479名(7.1%)の供血者が抗HEV IgG陽性であった。ALT≧201 IU/L群はHEV RNA有病率が有意に高かった。ウイルス血症を発症した供血者9名から得られたHEV分離ウイルスは遺伝子型3に分類された。ALT≧201 IU/Lの日本人の約3%はHEV株の無症候性感染を有することが示された。 | |

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| 70168 | 2007/12/20 | 70810 | E型肝炎 | Vox Sanguinis 2007; 93(Suppl.1): P203 | 2005年1月-2006年4月に北海道で献血者のHEV-RNAスクリーニングを行った。388,119名のうち、男性33名(1/7,120)、女性22名(1/6,962)がHEV-RNA陽性で、genotype 3が優勢であった。55名中40名は献血時のHEV抗体陰性であり、後に陽性となった。HEV陽性者にはALT値が上昇した人もいたが自覚症状はなかった。HEV-RNAは献血後、最長37日間検出された。HEV陽性献血者由来の輸血を受けた患者7名のうち、少なくとも2名が感染した。 | |
| 70168 | 2007/12/20 | 70810 | E型肝炎 | 肝臓 2007; 48(Suppl.1): O-178 | 発症前からのウイルス血症の推移、肝炎発症から沈静化までの経過を観察しえた輸血後E型肝炎2例の症例報告である。1例は輸血21日目にHEV RNA (genotype 4)が検出され、44日目にピーク値を、もう1例は輸血後3日目にHEV RNA (genotype 3)が同定され、54日目にピーク値を示した。HEVウイルス血症は潜伏期間を経て発現し、対数増殖後約50日前後にピークを示し、その直後にAST、ALT上昇と血中抗HEV抗体の出現を順に認めた。 | |
| 70171 | 2007/12/25 | 70823 | E型肝炎 | 第55回日本ウイルス学会学術集会 2P207 | HEVに感染したブタ糞便より精製した4種のHEVは、ウイルス除去膜PLANOVA15Nおよび20Nで全て検出限界以下にまで除去された。液状加熱実験では、PBS組成では加熱開始後短時間で全て検出限界以下となったが、アルブミン存在下では4株とも加熱開始後5時間目でも検出された。HEVは熱に弱いと考えれられていたが、条件によって不活化効果が異なることから、血液製剤や加工食品において慎重に不活化効果を検討しなければならない。 | 4 |
| 70169 | 2007/12/20 | 70811 | HIV | Clin Infect Dis 2007; 45: e68-71 | ボツワナで急性HIV-1感染スクリーニング中に特定された抗体陰性のHIV-1サブタイプC感染の初の症例を報告する。HIV-1抗体検査の結果は、迅速検査、通常の酵素免疫測定法及びウエスタンブロットで全て陰性であった。遺伝子組換えがないHIV-1サブタイプC感染は、ウイルスのgag、pol及びenv遺伝子のジェノタイピングによって確定された。臨床的に安定した状態からAIDS関連死までの期間は約3ヵ月だった。サブタイプCが優勢なアフリカ南部における血清学検査陰性HIV-1感染の調査の重要性が示された。 | |
| 70168 | 2007/12/20 | 70810 | HIV | Eurosurveillance 2007; 12(5): E070524.5 2007年5月24日 | AIDS最新号において、LikataviciusらはEuroHIV surveillance network によるヨーロッパの供血血液のHIV陽性率についての14年間のモニタリングデータを提示した。この分析は、1990-2004年のWHO欧州地域のデータが網羅されている。2000-2004年の10万供血中の平均HIV陽性率は西欧1.7、中欧3.4、東欧36.7であった。1990年以降の変化では、西欧で低下、中欧で横ばい、東欧では急激な上昇が認められた。 | |
| 70168 | 2007/12/20 | 70810 | HIV感染、C型肝炎、B型肝炎 | 第31回日本血液事業学会総会 2007年10月 3-5日 シンポジウム 4-2 | 日本赤十字社血液事業本部が関わる安全対策の取り組みと感染症リスクについて報告する。平成16年から18年までの3年間に全国の医療機関から日赤血液センターに報告された輸血関連感染症(疑い症例を含む)の報告数は749例であった。日赤の安全対策の実施によりHBV、HCV及びHIVの感染リスクは減少し、安全性は高くなった。しかし、HCV及びHIVも含め遡及調査の実施により確認された感染症例も少なくない。感染拡大を防止するための安全対策を引き続き講じていく必要がある。 | |
| 70168 | 2007/12/20 | 70810 | ウイルス感染 | CDC Press Release 2007年8月22日 | 米国疾病対策予防センター(CDC)と協力施設の科学者がよく見られるアフリカフルーツコウモリの一種でマールブルグウイルス感染を特定することに初めて成功した。マールブルグウイルスは、ヒトや霊長類に重篤で死に至ることも多い出血熱を引き起こす。コウモリがマールブルグウイルスを保有することが疑われていたが、証拠はなかった。この研究結果はPlos ONEに掲載された。この研究は、マールブルグウイルスの伝播についてより理解し、ヒトにおける感染拡大を予防・減少させる助力になると思われる。 | |
| 70168 | 2007/12/20 | 70810 | ウイルス感染 | J Clin Microbiol 2007; 45: 3008-3014 | ヨーロッパでの出血熱は主にPuumalaウイルス(PUUV)またはDobravaウイルス感染による。ドイツ南東部Lower Bavariaでハンタウイルス感染患者31名について、酵素免疫測定法、免疫蛍光法、免疫ブロット法による診断を行った。標準的検査による抗体のPUUV特異的タイピングができない症例が2、3あった。3名の患者の急性期血清から得たPUUV RNAをRT-PCR を用いて増幅したところ、同地域で捕獲したハタネズミ から得たウイルス配列と非常に近縁であることが明らかとなった。 | |
| 70171 | 2007/12/25 | 70823 | ウイルス感染 | Proc Natl Acad Sci 2007; 104: 11424-11429 | マレーシアMelakaで、高熱と急性呼吸器疾患に罹っていた39歳男性から未知のreovirusが分離され、Melaka virusと名づけられた。患者の家族も発症したが、この家族は発症前にコウモリと接触していた。遺伝子配列分析により、Melakaウイルスは1999年に同国Tioman島のフルーツコウモリから分離されたPulauウイルスと密接な関係があることが示された。同島住民の血清スクリーニングで、109例中14例(13%)が両ウイルスに陽性であった。 | |

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| 70168 | 2007/12/20 | 70810 | ウイルス感染 | ProMED-mail 20070702.2108 | 2007年6月22日、ミクロネシアのヤップ保健局で集められた血液検体をCDCの研究所で検査した結果、ヤップでの最近の疾病はジカウイルスが原因らしいことが示された。ヤップのアウトブレイクは2007年4月に始まり、5月後半にピークに達し、現在も続いている。症状は斑点状丘疹、結膜炎、関節痛など軽症で、4-7日間続く。6月29日現在、42例がPCRとIgM分析によってジカウイルス感染と確定された。死亡例はない。 | |
| 70168 | 2007/12/20 | 70810 | ウイルス感染 | ProMED-mail20070930.3228 | オーストラリアQueensland州で蚊が異常発生し、ロスリバーウイルスが拡大している。通常は北部の熱帯地域で優勢であるが、Brisbane南部における過去4週間の感染者数は、昨年(2006年)同時期のほぼ450%である。Queensland保健局の発表によると、過去4週間に報告された感染者数は93例であった。 | 5 |
| 70168 | 2007/12/20 | 70810 | ウイルス性脳炎 | Neurology 2007; 69: 156-165 | 同種造血幹細胞移植(HSCT)後に急性大脳辺縁系脳炎を発症した患者9名の臨床、EEG、MRI、ならびに臨床検査特性を調べた。患者は、順行性健忘、不適切な抗利尿ホルモン分泌症候群、軽度CSF多球症、一時的なEEG異常を特徴とした。MRIでは、T2、FLAIR、DWI画像にて、鉤、扁桃体、内側嗅領、海馬領域内に高信号域を認めた。PCRを用いた初回腰椎穿刺CSFの検査では9名中6名がHHV6陽性であり、同脳炎はHHV6と関連がある可能性が示唆された。 | |
| 70168 | 2007/12/20 | 70810 | ウエストナイルウイルス | The New York Times 2007年7月26日 | 米国におけるウエストナイルウイルス症例数は1年前の約4倍であり、大流行がおこる可能性があると政府研究者が報告している。昨年は米国で4,269症例が報告され、この中には1,495例の脳症が含まれ、177人が死亡した。今年はこれまで122症例が報告され、カリフォルニア州と南北ダコタ州で最も多いが、昨年の同時期は33例のみであった。今年は既に脳症が42例および死亡が3例ある。 | |
| 70168 | 2007/12/20 | 70810 | ウエストナイルウイルス | 第144回日本獣医学会学術集会 2007年9月2-4日 | 近い将来、日本にも侵入する可能性があるため、日本産蚊の室内継代株を用いてウエストナイルウイルス増殖・媒介能を調べた。アカイエカ、ヒトスジシマカ、オオクロヤブカでウイルス注入実験を、アカイエカ、ヒトスジシマカで吸血実験をしたところ、全種類の蚊においてウイルスの増殖が観察された。媒介試験では、アカイエカ注入、吸血両群、ヒトスジシマカ2系統の注入群、1系統の吸血群では供試したすべてのマウスが12日以内に死亡し、死亡したマウスからはWNV が検出された。 | |
| 80010 | 2008/01/29 | 70861 | エボラ出血 | CDC 2008年1月8日 | CDCとウガンダ保健省は、2007年8月から始まったウガンダ西部に位置するBundibugyo地区におけるエボラ出血熱のアウトブレイクを報告した。2008年1月3日までに148人が罹患し、37人が死亡した。患者検体の遺伝子解析により、既知の4つのエボラウイルス株と異なる、新たなウイルス株である可能性が示唆された。確定には更なる研究が必要である。 | 6 |
| 70169 | 2007/12/20 | 70811 | クロイツフェルト・ヤコブ病 | 2007年プリオン研究会 Poster-20 | 日本の人口動態統計では、CJDによる死亡は過去20年以上に渡り増加傾向を示し、2005年は人口100万対1.23人であった。CJDサーベイランス委員会による調査では過去8年間に918例がプリオン病と判定された。病型別では、孤発性CJD 716例、遺伝性プリオン病 128例、感染性(獲得性)CJD 72例(変異型CJD 1例、硬膜移植後CJD 71例)、および分類不能 2例であった。 | 7 |
| 70168 | 2007/12/20 | 70810 | チクングニヤウイルス感染 | Eurosurveillance 2007; 12(9): E070906.1 | チクングニヤ熱は2005年以来、大規模な流行がインド洋諸島とインドから報告されているが、これまでヨーロッパ地域内での蚊による感染伝播は発生していなかった。2007年8月にイタリアのエミリア・ロマーニャ州ラヴェンナ県衛生当局は異常に多数の発熱患者発生を検知し、臨床・疫学調査を行った。血清学的検査およびPCR法でチクングニヤ熱と確定された。更にヒトスジシマカからもPCR法によりチクングニヤウイルスが確認された。2007年9月4日までに合計197名の患者が報告されている。 | |
| 70168 | 2007/12/20 | 70810 | チクングニヤウイルス感染 | Pediatr Infect Dis J 2007; 26: 811-815 | チクングニヤウイルス感染が大流行したレユニオン島の5つの新生児医療部門で同ウイルスの母子感染を調べるため、後ろ向き記述的研究を実施した。母親は出産時に徴候があったか又は新生児が出生初日に発病したかをスクリーニングし、新生児38名を登録した。無症候の2名を除き、全母親が周産期 (分娩4日前～1日後)に症状があった。全新生児が発熱(79%)、疼痛(100%)などの症状を示し、脳脊髄液のPCR法は24名中22名で陽性であった。高い罹患率の周産期母子伝播の可能性が初めて示された。 | |

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | 新出文献No. |
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| 80010 | 2008/01/29 | 70861 | チクングニヤウイルス感染 | PLoS Pathogens 2007; 3: 1895-1906 | 2005～2006年にレユニオン諸島でアウトブレイクしたチクングニヤウイルス(CHIKV)感染は、エンベロープ蛋白遺伝子の変異株(E1-A226V)が関係していた。この変異の、ネッタイシマカおよびヒトスジシマカにおけるCHIKV適合性に対する影響を調べた。その結果、CHIKVのヒトスジシマカに対する感染性が有意に増加し、哺乳マウスへの伝播がより効率的になることが明らかとなった。通常のベクターであるネッタイシマカがいない同地域でCHIVKが大流行したのはこの変異が原因と考えられる。 | 8 |
| 70168 | 2007/12/20 | 70810 | デング熱 | ABC Newsletter 2007年7月20日 | オーストラリアのクイーンズランド州北部におけるデング熱アウトブレイクにより、赤十字血液サービスは流行地に滞在した人から供血された血液製剤の廃棄を余儀なくされた。局地的アウトブレイクは3月末にSouth Townsvilleで始まったが、5月14日以降、新規症例は記録されていない。赤十字のスポークスマンによると、供血以前に同地を訪れた供血者由来の赤血球・血小板は破棄するが、血漿は使用できるとのことである。供血制限は、アウトブレイクの終息が正式に宣言されるまで継続される。 | |
| 70166 | 2007/12/17 | 70803 | デング熱 | CDC/MMWR 2007; 56(31): 785-789 | 2005年7月にデング出血熱(DHF)症例1例がTexas州Brownsvilleの住民において報告された。2005年8月に隣接するメキシコTamaulipas州の保健当局はデング熱症例1251例が発生しているデング熱のアウトブレイクを報告し、内223例(17.8%)がDHFであった。臨床的および疫学的調査の結果、同地域でのデング熱アウトブレイクに伴うDHF症例の割合は、2000－2004年はデング熱症例541例中20例(3.7%)であり、増加していることが明らかとなった。 | |
| 70168 | 2007/12/20 | 70810 | デング熱 | ProMED-mail20071001.3237 | 2007年9月30日、中国保健当局はFujian省Putian市で39例のデング熱症例が確定されたと発表した。ベトナムでは2007年9月24日時点で約68000人が感染し、内60名が死亡した。パキスタンでは2007年9月26日、Karachiで新たに22例のデング熱症例が報告された。ラテンアメリカとカリブ海諸国ではデング熱の最悪のアウトブレイクが起こっており、2007年になってから何十万もの人々が関節痛を訴え、約200人が死亡した。 | 9 |
| 70171 | 2007/12/25 | 70823 | デング熱 | Trans R Soc Trop Med Hyg 2007; 101: 738-739 | 日本人のデング熱患者(28歳、女性)の血漿サンプル中ではなく尿及び唾液中でデングウイルスを検出することに成功した。発症後7、14および25日目の血漿検体中で抗デングウイルス抗体は同定されたが、デングウイルス遺伝子は検出されなかった。発症後7、8および14日目の尿、ならびに7日目の唾液からデングウイルス1型遺伝子が検出された。現在の研究の結果は、尿及び唾液中のデングウイルス遺伝子の検出が有効な診断方法、特にウイルス性出血の子供の診断方法になりうることを示唆している。 | |
| 70168 | 2007/12/20 | 70810 | デング熱 | YAHOO!ニュース 2007年10月14日 | 台湾南部でデング熱が流行している。台南市当局によると2007年10月13日までに市内で511人の感染者が確認された。隣接する高雄市でも2つの区で集団感染が発生しており、感染の広がりは過去最大規模である。行政と軍が協力して大規模な蚊の撲滅作戦を展開する方針である。 | 10 |
| 70169 | 2007/12/20 | 70811 | トリパノソーマ症 | ABC Newsletter 2007年9月14日 | AABBはCDCからAABBシャーガス病バイオビジランスネットワーク強化をするための資金を受けている。2007年9月13日現在、710名の反復反応性供血者がT. Cruziに対する抗体の追加RIPA試験を行った結果、196名がRIPA陽性、486名が無反応で、残りは結果がまだ出ていない。13の検査所がシャーガスネットワークにデータを報告し、18の検査所が同ネットワークにアクセスしている。 | |
| 70169 | 2007/12/20 | 70811 | トリパノソーマ症 | CMAJ 2007;177: 242 | カナダ血液サービスは、2008年後半の血液製剤製造プロセス見直しの際に北緯49度以北では稀にしか見られないシャーガス病のスクリーニングを開始する。2種類のシャーガス病検査法がカナダ保健省の認可を待っている。供血血液の検査実施は、血小板製剤の製造を「バフィーコート」法に切替えてからとなる。メキシコや中南米では800万人～1,100万人がシャーガス病の保因者であり、毎年45,000人以上死亡している。カナダでは、これまでに輸血による感染が2例マニトバ州で発生した。 | |
| 70169 | 2007/12/20 | 70811 | トリパノソーマ症 | 第48回　日本熱帯医学会大会 12C-02 | 日本におけるラテンアメリカ人の慢性シャーガス病キャリアーからの献血についての対策を検討した。カーミC液(CPD液)を用いてT.Cruzi感染マウス血液を4℃にて1-21日間保存処理を行ったところ、マウスへの感染性は無処理のものと差異は無かったが、病原性はかなり減弱することが示された。しかし、T.Cruzi虫体はほとんどの白血球除去フィルターを通過した。現在の保存血液提供システムはシャーガス病の輸血感染防止には不十分であり、対策の改善が必要である。 | 11 |

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| 80007 | 2008/01/25 | 70855 | パルボウィルス | J Gen Virol 2007; 88: 2162-2167 | ヒト血漿プール中に新規のパルボウイルスPARV4とその変異株であるPARV5が存在することが最近示された。4株のPARV4と2株のPARV5のDNA配列を分析したところ、PARV5はPARV4と同様に2つのオープンリーディングフレームを持ち、PARV4とPARV5は92%近くのヌクレオチド相同性を示した。両者は密接な関係のあるジェノタイプであり、ジェノタイプ1と2(PRV5と呼ばれていたもの)から成るPARV4という1つのウイルス名を使用することを提案する。 | |
| 70169 | 2007/12/20 | 70811 | パルボウィルス | Transfusion 2007; 47: 1756-1764 | 米国の血液センター7施設において2000-2003年の期間に採取した5020名の供血者由来の保存血漿検体を高感度PCRスクリーニング法を用いてパルボウイルスB19 DNAについて検査した。B19 DNA陽性率は0.88%であった。DNA陽性検体の全てがIgG陽性で、23%がIgM陽性であった。IgM血清陽性率はDNA値と相関した。 | 12 |
| 70171 | 2007/12/25 | 70823 | パルボウィルス | Transfusion 2007; 47: 883-889 | 1993-1998年及び2001-2004年の間に製造された6つの第Ⅷ因子濃縮剤の284ロットについて、in-house NAT法によりパルボウイルスB19 DNAを測定し、抗B19 IgGも併せて測定した。その結果、B19 NAT非スクリーニング血漿から調製した製剤のB19 DNAの陽性率及びレベルは高かったが、製造方法が異なると、製品間で様々であった。血漿のB19 NATスクリーニングは、最終製品中のB19 DNAレベルを下げ、大半の例で検出限界以下とさせ、B19伝播のリスクを減少させた可能性がある。 | |
| 80008 | 2008/01/25 | 70856 | パルボウィルス | Transfusion 2007; 47: 1765-1774 | B19ウイルスの不活性化機構を調べた。熱または低PHによるB19Vの不活性化はカプシド分解によるものではなく、感染性ビリオンがDNA枯渇カプシドへ変換することによって起こった。DNA枯渇カプシドは感染性はないが、標的細胞に接着することは可能であった。Parvoviridaeの他のウイルスとの比較試験の結果、被殻状態でのB19V DNAの著しい不安定性が明らかとなった。B19Vが不活化処理に抵抗性が低いのはこのためと考えられる。 | 13 |
| 80002 | 2008/01/23 | 70844 | パルボウィルス | Vox Sanguinis 2007; 93: 341-347 | 過去30～35年間に製造された第Ⅷ因子製剤中にヒトパルボウイルスが存在するかを調べた。175ロットのうち28ロットがPARV4シークエンスを含み、その内2ロットにジェノタイプ1型及び2型の両方が存在した。最大ウイルス量は10^5copies/mL以上であった。PARV4陽性の第Ⅷ因子製剤の大部分は1970年代及び1980年代に製造されていた。B19Vは175ロット中70ロットで陽性であった。 | 14 |
| 70171 | 2007/12/25 | 70823 | ヒトポリオーマウイルス感染 | J Virol 2007; 81: 4130-4136 | ヒトの気道からの検体をウイルススクリーニングし、KIポリオーマウイルスと暫定的に名付けた未知のポリオーマウイルスを同定した。このウイルスは、遺伝子のearly領域では、他の霊長類のポリオーマウイルスに系統遺伝学的に近縁であるが、late領域では、既知のポリオーマウイルスに対して相同性が少ない(アミノ酸同一性30%未満)。このウイルスは、PCRによって、鼻咽頭吸引物637例中6例(1%)と便検体192例中1例(0.5%)で検出されたが、尿及び血液検体では検出されなかった。 | |
| 70171 | 2007/12/25 | 70823 | ヒトポリオーマウイルス感染 | PLoS Pathogens 2007; 3: 595-604 | 急性呼吸器感染症に罹った患者からの呼吸分泌物中に存在する新規のポリオーマウイルスを同定し、WUウイルスと名付けた。WUウイルス遺伝子は5229bpで、Polyomaviridaeファミリーの特徴を持つ。系統遺伝学的分析から、このWUウイルスは、既知の全てのポリオーマウイルスとは異なっていることが明白となった。オーストラリア及び米国の急性呼吸器感染症患者2135例中43例からWUウイルスが検出され、地理的に広く分布していることが示唆された。 | |
| 70169 | 2007/12/20 | 70811 | マラリア | ABC Newsletter 2007年7月6日 | FDAは、初めて認証された米国のマラリア用迅速テスト、Binax NOWマラリア検査の使用を許可した。同検査は、非常に迅速で使用が簡便で、全血検体をディップスティックに2、3滴つけて15分後には結果が得られる。検査結果の確定には標準的顕微鏡検査法を用いなければならない。米国外のマラリア流行地域で行った多施設試験において、標準的顕微鏡診断と比較して当該検査の正確度は95%であった。 | |
| 70169 | 2007/12/20 | 70811 | マラリア | ProMED-mail20070501.1414 | ジャマイカ保健省によると、2007年4月の1ヶ月間に新規のマラリア症例11例が報告された。内2例はメスのハマダラカが媒介する熱帯熱マラリア原虫によるものであった。また、2006年12月に最初の症例が報告されて以降、輸入感染症例が7例あった。2007年4月1～21日の間に実施された884検体の検査の結果、血液検体陽性率は0.7～1.8%で減少を続けている。最近、Anopheles albimanus蚊がマラチオン殺虫剤に耐性を示し始めたことが確認されたため、感染拡大を防ぐために代わりの殺虫剤を探している。 | |

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| 70169 | 2007/12/20 | 70811 | マラリア | Vox Sanguinis 2007; 93(Suppl.1): P239 | 韓国における輸血によるマラリア感染の発生について調査した。マラリア診断前6ヶ月間以内に供血を行った供血者は、2005年5月～2006年8月の三日熱マラリア患者2056名中46名(2.2%)であった。46名の保管血液51検体についてPCRを実施し、PCR陽性の血液成分を輸血された全受血者を調査したところ、1名の受血者に輸血によるマラリア伝播が確認された。PCRに基づくマラリア遡及調査は、輸血によるマラリア伝播の特定に役立つ。 | |
| 70169 | 2007/12/20 | 70811 | リケッチア症 | Jpn J Infect Dis 2007; 60: 241-243 | 血清学的、微生物学的に確定された日本紅斑熱の初めての死亡症例を報告する。淡路島在住の77歳男性で、2005年9月2日に食欲低下を呈し、翌日、下腿に皮疹が出現、4日目に38.7℃の高熱、歩行障害、構音障害が出現、肝機能障害が急速に進行し、DIC、消化管出血により8日目に死亡した。右肩にダニ刺し口があった。血液よりDNAを抽出し、PCRを実施したところ、塩基配列はR. japonicaと100%一致した。日本紅斑熱は増加傾向にあり、注意が必要である。 | |
| 70169 | 2007/12/20 | 70811 | リケッチア症 | 朝鮮日報 2007年8月21日 | 韓国では最近ツツガムシ病の患者が急増している。2007年8月20日、疾病管理本部の発表によると、2002年に1,919人だったツツガムシ病の患者数が、04年は4,698人、06年には6,420人に増加したことが分かった。1993年末に法定伝染病に指定されて以来、患者数は実に25倍以上増加した。ツツガムシ病は、主に9月以降、ツツガムシ菌に感染したツツガムシ(ダニの一種)の幼虫に刺されることにより感染する。10日間程度の潜伏期を経ると、突然高熱が発生し、目の充血、頭痛、筋肉痛、発疹などの症状が現れる。 | |
| 80027 | 2008/02/27 | 70934 | ﾘﾝﾊﾟ性脈絡髄膜炎 | N Engl J Med 2008; 358 10.1056/NEJMoa073785 | オーストラリアで一人のドナーから臓器移植を受けた3例が移植後4-6週後に死亡した。他のいかなる方法でも原因不明であったが、2例のレシピエントの移植肝および腎から得られたRNAを公平な迅速シークエンシングで解析することにより、ﾘﾝﾊﾟ性脈絡髄膜炎に関係する新規のアレナウイルスが原因であることが明らかとなった。レシピエントの腎、肝、血液および脳脊髄液からこのウイルスが検出され、また免疫組織学的および血清学的に確認された。この方法は病原体発見の強力な手段である。 | 15 |
| 70169 | 2007/12/20 | 70811 | 異型クロイツフェルト・ヤコブ病 | 2007年プリオン研究会 Poster-38 | BSE感染ウシ由来の脳乳剤を用いてPrPresのin vitro感染系の確立を試みた。感染させたヒト由来グリオーマ細胞株から抗プリオン抗体に反応する約30KのPK耐性のバンドが検出された。このバンドは非感染細胞には存在しなかった。また、9ヶ月継代した感染細胞の培養上清に伝達性があることが明らかとなった。さらに20nmのウイルス除去膜によって培養上清の伝達性が減少することが認められた。 | 16 |
| 70168 | 2007/12/20 | 70810 | 異型クロイツフェルト・ヤコブ病 | ABC newsletter 2007年5月4日 | イスラエルで血液事業を行っているMagen David Adomは、変異型クロイツフェルト・ヤコブ病(vCJD)に関する供血延期基準を変更し、1980年以降にフランス居住歴がある人の供血を可能とした。1980年から10年間のうちにイギリス、アイルランド、ポルトガルに居住歴のある人は、引き続き供血禁止となる。また、輸血を受けた人、B型肝炎やC型肝炎患者と一緒に住んでいた人、入れ墨を入れた人、内視鏡検査を受けた人、未検査の動物に噛まれた人の供血延期期間を短縮した。 | |
| 70168 | 2007/12/20 | 70810 | 異型クロイツフェルト・ヤコブ病 | Biologicals 2007; 35: 79-97 | ドイツにおいて、vCJDが血液供給へ及ぼす影響について実際の集団データを基にモデル計算を行ったところ、輸血を介した伝播がvCJDを永続化するような可能性はなかった。更に、受血経験者を供血から排除しても輸血の安全性向上にはほとんど寄与しないが、血液供給には多大な影響を及ぼすと考えられた。そのためドイツにおいては受血経験者の除外は推薦されなかった。 | |
| 70171 | 2007/12/25 | 70823 | 異型クロイツフェルト・ヤコブ病 | Biologicals 2007; doi:10.1016/j.biologicals.2007.04.005 | 異なるポアサイズのウイルス除去膜を使用し、異なる処理を行ったスクレーピープリオン蛋白(PrPSc)の除去能力を評価した。超音波処理により粒子径分布を至適化するように調製した263K MFをスパイク物質として使用したときは、75nmのろ液中にPrPScが検出された。15nmのろ過のみが全ての条件でウエスタンブロット法の検出限界以下までPrPScが除去されることが示された。しかし、 1条件下の15nmろ液のバイオアッセイの結果では、感染性PrPScが確認された。 | |
| 70171 | 2007/12/25 | 70823 | 異型クロイツフェルト・ヤコブ病 | FDA/CBER 2006年11月7日 | 英国血漿由来の第XI因子製剤が、1989-2000年に米国で50名以下の患者に使用されたと推定される。モデルを用いたリスク評価の結果、1998年まで第XI因子製剤を製造するために使用された血漿プールの1.6%～50%がvCJD病原体を含んでいる可能性があった。しかし、これまで血漿由来製剤の投与を受けた患者において、世界中で一件もvCJDの症例は報告されていない。製造工程におけるvCJD除去、使用量、曝露経路および英国ドナーのvCJD有病率がリスクに影響を与える重要な因子である。 | |

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | 新出文献No. |
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| 70171 | 2007/12/25 | 70823 | 異型クロイツフェルト・ヤコブ病 | PLoS Pathogens 2007; 3: 659-667 | 経口的又は非経口的にスクレイピーを投与したハムスターの皮膚にPrPScが沈着するかを調べた。経口摂取したハムスターでは発症前にPrPScが検出され、発症時にはPrPScの蓄積がみられた。PrPScは皮膚の角化細胞ではなく神経線維に局在し、皮膚におけるPrPScの沈着は感染経路やリンパ組織感染に依存しなかった。神経が介在する遠心的な皮膚へのプリオン拡大が示された。更に、スクレイピーに自然感染したヒツジを調べたところ、5頭中2頭の皮膚検体中にPrPScが検出された。 | |
| 70168 | 2007/12/20 | 70810 | 異型クロイツフェルト・ヤコブ病 | Prion 2007 P04.102 2007年9月26-28日 | 1987年6月から1998年9月にかけて出荷された計175バッチの血漿製剤中に、後にvCJDと診断された11名からの供血が含まれていたが、これらの製品に関係したvCJD症例は今までのところ全く報告されていない。これは赤血球輸血によると思われるvCJD感染が3例あることと対照的である。血漿分画製剤の製造工程によるプリオン除去効果を調べたところ、2.7～11.5log以上の除去能があることが明らかとなった。 | 17 |
| 70168 | 2007/12/20 | 70810 | 異型クロイツフェルト・ヤコブ病 | Prion 2007; 2007年9月26-28日 Edinburgh P04.51 | 73歳の受血者で生前に特定されたvCJDの非典型的症状の報告である。患者は1997年12月に輸血を受けたが、供血後にvCJDを発症した供血者由来の赤血球製剤であった。輸血から6年後、受血者は疲労及び集中困難を訴えたが、神経学的検査及び脳MRIは正常であった。この6ヵ月後に神経学的症状が発現し、進行したが、血清学的検査は正常であった。MRIでは視床背側核全体の顕著な信号変化が示された。vCJDの長期潜伏期間と無症候状態は、重大な公衆衛生問題を提示する。 | |
| 70168 | 2007/12/20 | 70810 | 異型クロイツフェルト・ヤコブ病 | Proc Natl Acad Sci 2007; 104: 10998-11001 | アミロイドを含有するフォアグラにアミロイド促進因子(AEF)活性があるかを調べた。市販のフォアグラから抽出したアミロイドA蛋白含有フィブリルを、二次性アミロイドーシスを起こすトランスジェニックマウス9匹に静脈内投与したところ全例で、また経口投与した場合は8匹中5匹でアミロイドの組織沈着が見られた。一方、対照群では全く組織沈着は見られなかった。加熱によりフォアグラのAEF活性は弱まったが、消失しなかった。アミロイドーシスは伝播性で、プリオン関連疾患の感染性と類似する可能性がある。 | |
| 70168 | 2007/12/20 | 70810 | 異型クロイツフェルト・ヤコブ病 | SEAC/Position Statement 2007年6月13日 | 英国保健省はSEACに歯科治療処置を介したvCJD伝播のリスク概算を目的とした初期研究の知見についての助言を求めた。初期研究では、歯科処置によるvCJD伝播のリスクが予想より高いことが示唆された。ガイダンスは今年初め歯髄治療用器具の使い捨てを勧告した。公衆衛生上の影響についてのより綿密な考察と、さらなるリスク減少手段の特定のため、全ての歯科治療のリスクについて詳細で包括的な評価を早急に行うことも重要である。 | |
| 70171 | 2007/12/25 | 70823 | 肝炎 | Med Mol Morphol 2007; 40: 23-28 | ALTが高く、HCV抗体とB型肝炎表面抗原が陰性である供血者からの血漿検体中のウイルス様粒子(VLPs)を視覚的に捉えようと試み、また、このVLPsと非経口的に感染するGBV-C/HGVの遺伝子との関係を調べた。その結果、循環血液中のVLPsの検出率は、有意にALTレベル上昇と関係($P<0.001$)していたが、VLPsを含む血漿のいずれにも、GBV-C/HGV RNAは検出されなかった。電子顕微鏡で球状のVLPsが確認され、それらが非B非C型肝炎に関係していることが示唆された。 | |
| 80007 | 2008/01/25 | 70855 | 寄生虫感染 | Int J Med Microbiol 2007; 297: 197-204 | ドイツにおけるヒトバベシア症の初めての症例を報告する。患者は結節性リンパ球性ホジキンリンパ腫が再発し、脾臓摘出されたドイツ人の63歳男性で、リツキシマブ投与後、貧血とヘモグロビン尿による暗色尿のため入院した。末梢血塗抹標本で梨状の寄生虫赤血球封入体が確認されバベシア症と推定され、Babesia特異的18S rDNA PCRによって確認された。シークエンス分析によりEU1と99.7%の相同性があり、EU3と名づけられた。寄生虫が消えるまでにはatovaquoneによる長期治療を要した。 | |
| 70169 | 2007/12/20 | 70811 | 結核 | Emerg Infect Dis 2007; 13: 380-387 | 第二選択抗結核剤6クラスのうち3つ以上に耐性を示す多剤耐性結核を広範囲薬剤耐性結核(XDR TB)と定義し、2000年～2004年のSupranational Reference Laboratoriesのネットワークを調査した。48カ国からのMycobacterium tuberculosis分離株17,690のデータが提供され、多剤耐性分離株3,520のうち、347(9.9%)がXDR TBであった。 | |
| 70169 | 2007/12/20 | 70811 | 細菌感染 | ABC Newsletter 2007年9月21日 | FDAは輸血前の血小板中の細菌汚染を検出するための初めての迅速検査を販売承認した。Verax Biomedical Inc 製造のPlatelet Pan Genera Detection Test Systemは病院の輸血部で使用するための使い捨て検査機器である。 | 18 |

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| 70169 | 2007/12/20 | 70811 | 細菌感染 | American Society for Microbiology 107th Annual Meeting; L-004 2007年5月21－25日 | 日本の三次医療施設である自治医科大学病院（病床数1082床）において、2006年4月1日～8月31日に、患者28名の血液培養からBacillus cereusが検出された。リネン類の汚染と末梢静脈ラインの不適切な取り扱いが原因であると考えられた。一時的にリネン類のオートクレーブ処理を行い、洗濯機を洗浄し、末梢静脈ライン管理について職員の教育を行ったことで、B. cereus陽性血液培養はその後検出されなかった。 | |
| 70169 | 2007/12/20 | 70811 | 細菌感染 | Clin Infect Dis 2007; 44: 1408-1414 | 2005年3月、米国ネブラスカ州の病院で複数の病室において、無針静注カテーテルコネクターバルブが導入された時期に血流感染の急激な増加が見られた。一次血流感染について調査を行ったところ、一次血流感染と無針静注カテーテルコネクターバルブの使用との間に有意な関連性が認められた。細菌培養を行った37個のバルブのうち24.3%から微生物が検出され、主にコアグラーゼ陰性ブドウ球菌であった。無針コネクターバルブの評価には市場導入前に感染リスクの査定を含めるべきである。 | |
| 70169 | 2007/12/20 | 70811 | 細菌感染 | Transfusion 2007; 47: 1134-1142 | アメリカ赤十字で2004年3月1日～2006年5月31日の期間に1,004,206例の供血で細菌培養検査が行われ、その内186例が陽性であった。関連するアフェレーシス血小板293製剤のうち1件を除くすべての輸血が回避された。両腕法を用いて採取した場合の細菌培養陽性率は、片腕法と比較して有意に高かった。また、スクリーニング陰性の製剤に関係した敗血症性輸血反応が20例（うち死亡3例）報告されたが、両腕法を用いて採取した場合の頻度は片腕法と比較して4.7倍であった。 | |
| 80024 | 2008/02/22 | 70921 | 人畜共通感染症 | Vet Microbiol 2004; 104: 113-117 | 異なった地域のﾌﾞﾀから収集された血清検体のうち66.2%(102/154)でﾌﾞﾀTTｳｲﾙｽDNAが検出された。ﾌﾞﾀTTｳｲﾙｽ自体はﾌﾞﾀで発現する疾患との関連は知られていないが、他の病原体と共感染した場合に疾患を増悪させる可能性は否定できない。ﾌﾞﾀ臓器などを使用した異種移植の際のﾋﾄへの影響が懸念される。 | |
| 80026 | 2008/02/27 | 70933 | 鳥インフルエンザ | China View, www.chinaview.cn 2008-01-10 | 2007年12月に江蘇省南京で発生した52歳男性の鳥インフルエンザ感染患者は、患者であった息子との濃厚な接触により感染したものであり、ウイルスの変異は認められていない。しかし、息子と父親はいずれも死亡した家禽との接触がないため、息子の感染源は明らかになっていない。息子は11月24日に発症し、12月2日に死亡し、父親は12月3日に発症したが回復した。ヒト用トリインフルエンザワクチンは臨床試験Phase IIの段階にある。 | 19 |
| 70171 | 2007/12/25 | 70823 | 鳥インフルエンザ | Emerg Infect Dis 2007; 13: 1081-1083 | 高病原性鳥インフルエンザウイルス(H5N1)を含むインフルエンザウイルスが、血液安全性の脅威となるおそれがある。ミニプール核酸増幅法を用いて10,272例の血液ドナー検体を分析した。この検査法の測定感度は、一般的インフルエンザウイルス用プライマーについては804 geq/ml、インフルエンザ(H5N1)サブタイプ特異的プライマーでは444 geq/mlであった。インフルエンザウイルスに対して、このようなスクリーニング検査が可能であることが示された。 | |
| 80001 | 2008/01/11 | 70841 | 鳥インフルエンザ | Emerg Infect Dis 2007; 13: 1348-1353 | 2006年5月にインドネシアのスマトラ北部でおよび2005年12月にトルコ東部の家族で観察されたトリインフルエンザH5N1の集団が、ヒト-ヒト伝播によるか否かを統計的方法を用いて調べた。スマトラの例ではヒト-ヒト伝播の統計学的エビデンスが見られ、概算された2次感染率は29%、局所的増殖数の下限値は1.14であった。トルコの例ではヒト-ヒト伝播のエビデンスは得られなかった。 | |
| 80001 | 2008/01/11 | 70841 | 鳥インフルエンザ | Lancet 2007; 370: 1137-1145 | H5N1インフルエンザウイルスに感染した男性1名および妊婦1名とその胎児の剖検組織を調べた。肺のⅡ型上皮細胞、気管の上皮細胞、リンパ節のT細胞、脳の神経細胞及び胎盤のホフバウエル細胞と細胞栄養層でウイルス遺伝子配列と抗原が検出され、腸粘膜ではウイルス遺伝子配列のみが検出された。胎児では肺、末梢単核細胞、肝マクロファージに遺伝子配列と抗原が検出された。本ウイルスは肺だけでなく気管に感染し、脳を含む他の器官に拡がり、また胎盤を通過し、母親から胎児にも伝播しうる。 | |
| 70168 | 2007/12/20 | 70810 | 日本脳炎 | Epidemiol Infect 2007; 135: 974-977 | 2004年11月から2005年2月にかけて、日本の西部に位置する広島県の野生イノシシから血清25検体を採取した。日本脳炎ウイルス（JEV）に対する抗体検査を、IgMキャプチャー及びIgG酵素免疫測定法（ELISA）、並びにプラーク減少中和試験により行った。17検体（68%）がJEV中和抗体陽性だった。中和抗体陽性検体は全てIgG-ELISA陽性だった。1検体はIgMも陽性だった。約70%の野生イノシシが抗JEV抗体陽性であることが示され、この地域のJEV感染サイクルに関与している可能性が提示された。 | |

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| 70168 | 2007/12/20 | 70810 | 麻疹 | asahi.com 2007年4月18日 | 東京都や埼玉県など関東地方ではしかが流行していることが、国立感染症研究所感染症情報センターがまとめた定点調査でわかった。例年より流行は早く、人の移動が活発になる連休に向けてさらに広がることが予想されるとして、同センターは緊急情報を出して注意を呼びかけている。同センターによると、例年、はしかの発症は乳幼児に多いが、今年の流行は10代前半や大人に多いのが特徴という。 | |

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| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2007 年 10 月 21 日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | ①ポリエチレングリコール処理抗破傷風人免疫グロブリン<br>②乾燥抗破傷風人免疫グロブリン | | 研究報告の公表状況 | 第 55 回日本ウイルス学会学術集会<br>2P213 | 公表国<br>日本 | |
| 販売名<br>（企業名） | ①テタノブリン－IH（ベネシス）<br>②テタノブリン（ベネシス） | | | | | |

**研究報告の概要**

【目的と意義】
A 型肝炎ウイルス (HAV) はエンベロープがなく、血液製剤の不活化処理で不活化されにくい。外国では血液製剤を介して HAV に感染した事例があり、製造工程における安全対策上重要なウイルスである。一般に血液製剤の Validation 試験では HAV 細胞馴化株が使用されるが、馴化株には多くの遺伝子変異が見られることから、株間で不活化処理に対する感受性の異なる可能性がある。既に我々は、熱抵抗性に変化の生じることを報告した (戸塚敦子等、第 45 回ウイルス学会)。今回、Validation 試験に適切な株を選別することを目的として、血液製剤の製造工程でよく使われている加熱処理と、広範囲の病原体不活化が期待される新規不活化法である加圧処理とを試み、遺伝子型の違う複数の HAV 細胞馴化株間で不活化効果に差異があるか検討した。

【材料と方法】
HAV 細胞馴化株は、KRM238、KRM003 (遺伝子型 IIIB)、KRM031 (IA 型)、TKM005 (IB 型) を使用した。ウイルス感染価は、HAV に感染した GL37 細胞を免疫染色法にて測定した。加熱処理は、25%アルブミンに HAV を添加し 60℃で 1～10 時間加熱した。加圧処理は、室温下で 300～420MPa の 1 分間加圧の後減圧するサイクルを 3 回繰り返した。

【結果】
60℃ 10 時間加熱処理において、KRM003 と KRM031 は約 5log の感染価低下が認められたのに対し、他の 2 株は約 3log の低下であった。また 420MPa 加圧処理において・KRM031 は約 5log の低下が認められたのに対し、他の 3 株は約 3log の低下であった。加熱処理と加圧処理の其々に不活化され易い株とされにくい株があり、両者で約 2log の差異があった。

【考察】
HAV 細胞馴化株間で不活化効果に差異があることを明らかにした。TKM005 は他株より細胞で増えにくいことを考慮すると、Validation 試験に使用する株として、加熱や加圧で不活化されにくく細胞で良く増殖する KRM238 が適切であると考えられた。また、血液製剤の製造工程に新規不活化法を導入する場合には、従来法で不活化されにくい株が、新規不活化法にも抵抗性であるとは限らないため、Validation 試験に使用する株を適切に選定して不活化評価を慎重に行う必要がある。

| 報告企業の意見 | 今後の対応 |
|---|---|
| HAV 細胞馴化株間で液状加熱および加圧による不活化効果に差異があることを明らかにしたとの報告である。<br>本剤からHAVが伝播したとの報告はない。万一HAVが混入したとしても、EMC及びCPVをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程において十分に不活化・除去されると考えている。 | モデルウイルスを用いたウイルスバリデーション試験に加えて、必要に応じて実ウイルスを用いた工程評価を実施する。 |

**使用上の注意記載状況・その他参考事項等**

代表としてテタノブリン－IH の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の破傷風抗毒素を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

## 2P212

## Axonal degeneration as a self-destructive defense mechanism against neurotropic virus infection

角田郁生
ユタ大学　医学部　神経内科
itsunoda@hotmail.com

【Introduction】
Until recently, it was believed that, following axonal injury, the disconnected axon degenerates as a consequence of a lack of trophic support from the cell body. The findings in C57BL/Wld$^S$ (Wld$^S$, Wallerian degeneration slow mutant) mice suggest that axonal degeneration is an active program of self-destruction in many physiological and pathological settings. Wld$^S$ mice are protected from axonal (Wallerian) degeneration by overexpression of a fusion protein (Wld$^S$). Although preservation of axons is beneficial in most cases, delay of axonal degeneration can be detrimental in infection with viruses that use axonal flow for their spread in the central nervous system (CNS).
【Materials and Methods】
We infected Wld$^S$ mice and their parent strain C57BL/6 (B6) mice with Theiler's murine encephalomyelitis virus (TMEV), a virus that can be transported by axonal flow. Virus persistence and neuropathology were examined 1, 2, 3, 5 weeks and 3 and 6 months after infection.
【Results】
B6 mice are known to be relatively resistant to TMEV infection, and a small percentage of mice developed paralysis. In contrast, 30 % and 50 % of Wld$^S$ mice showed limb paralysis during the acute and chronic stages of infection, respectively. Wld$^S$ mice had prolonged inflammation and larger numbers of viral antigen containing cells in the CNS, compared with B6 mice. Despite the protection from axonal degeneration, Wld$^S$ mice had neuronal death (apoptosis) and marked loss of MAP-2 immunoreactivity, suggesting that apoptosis and dendritic pathology cannot be prevented by the *Wld$^S$* gene.
【Discussion】
Prolonged survival of axons in Wld$^S$ mice could favor virus spread in the CNS, while axonal degeneration in B6 mice might be a beneficial self-destructive mechanism that limits the spread of virus in the CNS. Thus, neurons seem to have two self-destruction programs: apoptosis and axonal degeneration, both of which can limit neurotropic virus propagation in the nervous system.

## 2P213

## 加熱および加圧によるA型肝炎ウイルスの不活化 －株間の差異の検討－

嶋崎典子[1]、清原知子[2]、戸塚敦子[2]、梅森清子[3]、
岡田義昭[3]、米山徹夫[2]
財団法人北里環境科学センター　ウイルス部[1]、
国立感染症研究所　ウイルス第二部[2]、
国立感染症研究所　血液・安全性研究部[3]
shimasaki@kitasato-e.or.jp

【目的と意義】
A型肝炎ウイルス（HAV）はエンベロープがなく、血液製剤の不活化処理で不活化されにくい。外国では血液製剤を介してHAVに感染した事例があり、製造工程における安全対策上重要なウイルスである。一般に血液製剤のValidation試験ではHAV細胞馴化株が使用されるが、馴化株には多くの遺伝子変異が見られることから、株間で不活化処理に対する感受性の異なる可能性がある。既に我々は、熱抵抗性に変化の生じることを報告した（戸塚敦子等、第45回ウイルス学会）。今回、Validation試験に適切な株を選別することを目的として、血液製剤の製造工程でよく使われている加熱処理と、広範囲の病原体不活化が期待される新規不活化法である加圧処理とを試み、遺伝子型の違う複数のHAV細胞馴化株間で不活化効果に差異があるか検討した。
【材料と方法】
HAV細胞馴化株は、KRM238、KRM003（遺伝子型IIIB）、KRM031（IA型）、TKM005（IB型）を使用した。ウイルス感染価は、HAVに感染したGL37細胞を免疫染色法にて測定した。加熱処理は、25%アルブミンにHAVを添加し60℃で1～10時間加熱した。加圧処理は、室温下で300～420MPaの1分間加圧の後減圧するサイクルを3回繰り返した。
【結果】
60℃10時間加熱処理において、KRM003とKRM031は約5log$_{10}$の感染価低下が認められたのに対し、他の2株は約3log$_{10}$の低下であった。また420MPa加圧処理において、KRM031は約5log$_{10}$の低下が認められたのに対し、他の3株は約3log$_{10}$の低下であった。加熱処理と加圧処理の其々に不活化され易い株とされにくい株があり、両者で約2log$_{10}$の差異があった。
【考察】
HAV細胞馴化株間で不活化効果に差異があることを明らかにした。TKM005は他株より細胞で増えにくいことを考慮すると、Validation試験に使用する株として、加熱や加圧で不活化されにくく細胞で良く増殖するKRM238が適切であると考えられた。また、血液製剤の製造工程に新規不活化法を導入する場合には、従来法で不活化されにくい株が、新規不活化法にも抵抗性であるとは限らないため、Validation試験に使用する株を適切に選定して不活化評価を慎重に行う必要がある。

# 医薬品　研究報告 調査報告書

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| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| 抗 HCV 抗体陽性の慢性肝炎患者では GOR 自己抗原決定基に対する抗体反応がしばしば検出される。<br>本研究は、潜在性 HCV 感染患者の血清中に抗 GOR 抗体が検出されるかどうかを調査し、潜在性 HCV 感染における抗 GOR 抗体アッセイの診断的意義を評価することを目的とした。<br>肝組織における HCV RNA の検出によって HCV 感染があると診断され、血清中の抗 HCV 抗体は陰性である潜在性 HCV 感染患者 110 例から得られた血清を対象に、抗 GOR 抗体の反応性を検討した。抗 GOR 抗体は 110 例中 22 例（20%）で検出され、その頻度と力価は慢性 C 型肝炎患者（70/110、63.6%）と比較して有意に低かった（P<0.001）。HCV とは無関係の肝疾患患者 120 例ではいずれも抗 GOR 抗体は検出されなかった。<br>潜在性 HCV 感染患者の臨床、検査および組織学的特徴は、HCV RNA 陽性肝細胞の割合が抗 GOR 抗体陽性の潜在性 HCV 感染患者で有意に高かった（P=0.042）。このことを除き、いずれも抗 GOR 抗体の状態による差は認められなかった。<br>結論として、市販の検査では抗 HCV 抗体が検出されなくとも、潜在性 HCV 感染患者には抗 GOR 抗体が存在する。血清に HCV RNA が検出されない患者において抗 GOR 抗体の検査を行うことは、肝生検を実施することなく、少なくとも一部の潜在性 HCV 感染を検出する上において有用と思われるが、正確に診断するためには大多数の患者では肝生検が必要になると思われる。 | |

| 報告企業の意見 | 今後の対応 |
|---|---|
| 血清中から抗 HCV 抗体は検出されず、肝生検により HCV RNA の検出される潜在性 HCV 感染の一部(20%)を抗 GOR 抗体検査の実施により検出することができるとの報告であるが、現時点で本検査法に対する正確性、有用性の評価はできない。<br>弊社の血漿分画製剤の製造工程には二つ以上のウイルス除去・不活化工程が組み込まれており、最終製品において核酸増幅検査により HCV RNA が陰性であることを確認している。 | 今後とも HCV に関する情報に留意していく。 |



(2)

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# Serum IgG Antibodies to the GOR Autoepitope are Present in Occult Hepatitis C Virus (HCV) Infection Despite Lack of HCV-specific Antibodies

Juan A. Quiroga, Inmaculada Castillo, Javier Bartolomé and Vicente Carreño*.

Fundación para el Estudio de las Hepatitis Virales. Madrid, Spain.





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Key words: hepatitis C virus (HCV); seronegative occult HCV infection; serological diagnosis; IgG anti-GOR testing

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*Corresponding author: Vicente Carreño, MD

Fundación para el Estudio de las Hepatitis Virales. Guzmán el Bueno, 72. 28015 Madrid, Spain. Tel:+34-91.544.6013; Fax; +34-91.544.9228; e-mail: fehvhpa@fehv.org

## ABSTRACT

*Antibody responses to the GOR autoepitope are frequently detected among anti-hepatitis C* virus (anti-HCV)-positive patients with chronic hepatitis. GOR antibody reactivity has been investigated in sera from 110 anti-HCV-negative patients with occult HCV infection as diagnosed by detection of HCV RNA in hepatic tissue. A positive test to IgG anti-GOR was found in 22 (20%) of them. The frequency and titres of IgG anti-GOR were significantly lower compared with chronic hepatitis C patients (70/110, 63.6%; P<0.001). IgG anti-GOR was not detected in any of the 120 patients with HCV-unrelated liver disease. The IgG anti-GOR assay showed values of specificity and sensitivity of 100% and 20%, respectively, among occult HCV-infected patient sera; the predictive values (positive, PPV; negative, NPV) were 100% and 44.3%. None of the clinical, laboratory and histological characteristics of the patients with occult HCV infection were different according to GOR antibody status except that the percentage of HCV RNA-positive hepatocytes resulted significantly greater (P=0.042) in patients with occult HCV infection who tested positive to IgG anti-GOR. In conclusion, serum IgG anti-GOR is present in patients with occult HCV infection despite lack of detectable HCV-specific antibodies using commercial tests. Testing for IgG anti-GOR in patients without HCV RNA detected in serum may help in identifying a subset of occult HCV infection without performing a liver biopsy.


Key words: GOR antibody reactivity; anti-HCV-negative occult HCV infection; chronic hepatitis C; host-derived antigen GOR; cross-reactivity.

## INTRODUCTION

Occult hepatitis C virus (HCV) infection has been described recently in patients with persistently abnormal liver function tests of unknown etiology (2). Occult HCV infection has been noted by other authors as well (4,27). Because such patients are repeatedly negative by current assays for antibodies to HCV and HCV RNA in serum, occult HCV infection is identified by detection of HCV RNA in hepatic tissue. Except for the serological profile, occult HCV infection show characteristics similar to those observed in patients with chronic hepatitis C. Thus, HCV RNA has been detected in peripheral blood mononuclear cells of a high percentage of patients (2). Also, HCV replication has been demonstrated in peripheral blood mononuclear cells from occult HCV-infected patients (3), in a similar way to patients with chronic hepatitis C. In addition, ultracentrifugation studies have revealed that the buoyant densities of HCV RNA from occult HCV-infected patients are comparable to those of particles found in the serum of patients with chronic hepatitis C (unpublished results). Furthermore, patients with occult HCV infection may potentially benefit from interferon-based therapies as reported recently (18).

The GOR (GOR47-1) gene product is a host-derived antigen isolated from a cDNA library of host animals (16) which cross-reacts on immunoassays with sera of hepatitis C virus-positive patients. The human counterpart of the GOR gene product has been isolated recently (8); its sequence was highly conserved compared with that of the chimpanzee. Antibodies against another GOR epitope (termed GOR1-125), which is translated in humans, have been detected in some individuals without association with HCV infection (8). The detection of antibodies to the GOR47-1 autoepitope (anti-GOR) was first described in sera from non-A, non-B hepatitis cases (16). Since then, several studies have

shown that the presence of anti-GOR is almost restricted to anti-HCV-positive individuals (14,15). The sequence of the GOR (GOR47-1) epitope has a partial homology with the HCV-encoded core protein sequence (17); both sequences show a high conservation of residues essential for antibody binding (34). Antibodies against GOR are frequently detected among patients with overt HCV infection (6,16,21,31). Thus, anti-GOR appears to be an antibody specifically related to HCV infection (15,16).

On the other hand, there is little evidence of a relationship between autoimmunity and GOR in human beings (13). However, because HCV infection may be associated with extrahepatic autoimmune disorders (20) such as cryoglobulinemia (5) and autoimmune hepatitis (15) the presence of serum factors associated with inflammatory conditions that could interfere with GOR-antibody detection needs to be ruled-out. Prior studies have found anti-GOR responses in a small percentage of individuals with chronic liver disease but without HCV RNA (28,33) but none of these have previously investigated the presence of occult HCV. Up to date there are no data reporting on the detection of anti-GOR in patients with occult HCV infection.

The aims of this work have been to investigate whether anti-GOR can be detected in the sera of occult HCV-infected patients and to assess the diagnostic significance of GOR-antibody assay in occult HCV infection.

## MATERIALS AND METHODS

**Study subjects.** One hundred ten patients with a diagnosis of occult HCV infection were enrolled in this study. They were serum anti-HCV-negative (Innotest-HCV Ab IV, Innogenetics, Gent, Belgium) and serum HCV RNA-negative (Amplicor HCV version 2.0; Roche Diagnostics, Branchburg, NJ; sensitivity of 50 IU/mL), and presented sustained abnormal liver function tests of unknown etiology for a minimum time of 12 months (tested every 3 months) prior to undergoing a liver biopsy for histological diagnosis (26) which demonstrated the presence of hepatic HCV RNA assayed by both PCR (110/110, 100%) and in situ hybridization (108/108 tested; 100%) as reported elsewhere (2). HCV RNA amplified from liver biopsies was genotyped by a standard method (Inno-LIPA HCV II, Innogenetics); all patients with occult HCV infection showed HCV1b (2). Other known causes of liver disease were excluded based on clinical, epidemiological and laboratory data: infection by HBV (hepatitis B surface antigen and serum HBV DNA negative), autoimmunity (negative for anti-nuclear and anti-mitochondrial antibodies, etc.), metabolic and genetic disorders, alcohol intake, drug toxicity, etc.; all subjects were negative for anti-HIV antibodies. There were no known risk factors for HCV infection; none of the patients referred clinical or biochemical history of acute hepatitis.

Control groups included: 110 patients with chronic hepatitis C (serum anti-HCV and HCV RNA-positive and abnormal transaminase values; all with HCV genotype 1); 35 patients with cryptogenic liver disease (serum anti-HCV and HCV RNA-negative and liver HCV RNA-negative but abnormal transaminase values); 35 patients with non-viral liver disease: 10 with autoimmune hepatitis, 10 with primary biliary cirrhosis, 5 with alcoholic hepatitis and 10 with steatosis or steatohepatitis (all were liver HCV RNA-negative); and

50 patients with chronic hepatitis B (all serum HBV DNA-positive: 15 hepatitis B e antigen-positive and 35 anti-HBe-positive). The study was approved by the ethics committee of the institution and was conducted according to the Declaration of Helsinki on human experimentation. Informed consent was obtained from the patients.

**Enzyme immunoassay to detect IgG anti-GOR.** A pentadecapeptide with the sequence GRRGQKAKSNPNRPL corresponding to the GOR (GOR47-1) epitope (16) was purchased from RayBiotech Inc. (Norcross, GA); the lyophilized peptide had a purity > 80% as determined by high-performance liquid chromatography. The peptide was dissolved and diluted to a concentration of 1 mg/ml in deionized ultrapure sterile water.

Detection of IgG antibody to GOR was done by enzyme immunoassay. In brief, wells of a 96-well microtitre EIA plate (Costar, Cambridge, MA) were coated with 10 µg/ml GOR peptide in 0.1 M sodium carbonate buffer pH 9.6 for 18 h at 4 °C. Wells were washed with PBS pH 7.4 containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, MO) and non-specific sites blocked by incubating for 1 h at 37 °C with PBS containing 0.05% Tween 20 plus 10% heat-inactivated fetal bovine serum (Sera Laboratories International Ltd., West Sussex, UK). Serum samples were diluted 1:10 in blocking buffer and pre-incubated for 1 h at 37 °C with shaking; then, samples were allowed to react in duplicate with GOR-coated wells for 1h at 37 °C (100 µl/well) . Wells were washed five times as above and incubated (1 h at 37 °C) with horseradish peroxidase-conjugated rabbit polyclonal anti-human IgG (DakoCytomation A/S, Glostrup, Denmark) diluted 1:1000 in blocking buffer. After washing as above wells were reacted for 30 min at room temperature in the dark with 2,2'-azinobis-[3-ethylbenzthiazoline-6-sulfonic acid]-diammonium salt (ABTS; Pierce, Rockford, IL) and the absorbance value measured at 405 nm with a reference at 620 nm. A sample was considered reactive to IgG anti-GOR if the

absorbance value exceeded the mean absorbance values of 20 non-exposed, HCV-negative healthy volunteers plus five times the standard deviation. Typical cut-off values were below 0.11 absorbance units at 405/620 nm.

The specificity of the IgG anti-GOR antibody detection was assured by peptide inhibition assay as reported previously (21), in which serum samples were pre-incubated without or with the GOR peptide (10, 100 and 1000 μg/ml) in blocking buffer and then reacted in duplicate in the EIA as described above. A decrease of more than 50% in the absorbance values denoted inhibition of IgG anti-GOR detection. The reproducibility of the IgG anti-GOR assay was assessed in three separate runs, with the same samples from GOR IgG antibody-negative and -positive samples. The intra- and inter-assay coefficients of variation were 8.8% and 9.9%, respectively. Titration of IgG anti-GOR was done as described above in GOR IgG antibody-positive samples by serial two-fold serum dilutions (starting from 1:20) in blocking buffer and then reacted in duplicate in the EIA.

Determinations of rheumatoid factor and C-reactive protein were assayed in serum samples using latex agglutination tests (Biokit S.A., Barcelona, Spain). Presence of cryoglobulins was visually assessed by the appearance of a cryoprecipitate by blood coagulation at 37°C, centrifugation and incubation of serum at 4°C for 24 to 72 hours.

**Statistical analysis.** Results were analyzed by non-parametric tests using the SPSS program (version 9.0; SPSS Inc., Chicago, IL). The chi-square test (or Fischer's exact test when applicable) was used to compare frequencies. Correlations were done using the Spearman's rank correlation coefficient. All P values reported are two-tailed.

## RESULTS

Twenty-two of the 110 (20%) patients with occult HCV infection had IgG anti-GOR detectable in their serum. The specificity of the IgG anti-GOR antibody detection was demonstrated by peptide inhibition assay as shown in figure 1. Thus, pre-incubation of serum samples with the GOR peptide resulted in a decrease of more than 50% in the absorbance values of IgG anti-GOR detection, whereas less than 5% blocking was noted following pre-incubation with an irrelevant peptide.

In patients with chronic hepatitis C, 70/110 (63.6%) had serum IgG anti-GOR. Thus, the frequency of GOR antibody detection was significantly higher in patients with chronic hepatitis C compared with individuals with occult HCV infection ($P < 0.001$). IgG anti-GOR was neither detected in 35 patients with cryptogenic liver disease nor in 35 others suffering from non-viral liver diseases, irrespective of the etiology of the disease; similarly, IgG anti-GOR was undetectable in fifty chronic hepatitis B patients.

To assess the analytical performance of the IgG anti-GOR assay the sensitivity and specificity parameters were calculated with a threshold of detection set at 0.11 absorbance units as described in Materials and Methods. The "gold standard" to evaluate the accuracy of the IgG anti-GOR test was the presence of hepatic HCV RNA that had allowed identifying occult HCV infection. Thus, the IgG anti-GOR assay showed values of specificity and sensitivity of 100% and 20%, respectively, among occult HCV-infected patient sera. Similarly, the predictive values (positive, PPV; negative, NPV) were 100% and 44.3%, respectively, considering 70 HCV RNA-negative patients with HCV-unrelated non-viral liver disease.

Titration of IgG anti-GOR showed a median value of 1:20 in patients with occult HCV infection with serum GOR antibody titres ranging from 1:10 to 1:80 (figure 2). In patients with chronic hepatitis C the median IgG anti-GOR titre was 1:80 and titres ranged from 1:40 to 1:320. Thus, GOR IgG antibody levels were significantly lower among individuals with occult HCV infection compared with chronic hepatitis C patients ($P < 0.001$; figure 2). On the other hand, the analysis of IgG anti-GOR titres in sequential serum samples demonstrated minor changes in IgG anti-GOR levels among GOR antibody-positive patients with occult HCV infection. Similarly, there were no changes in IgG anti-GOR tires among GOR antibody-positive untreated chronic hepatitis C patients within a one-year period of survey (data not shown).

As regards the clinical, laboratory and histological characteristics, patients with occult HCV infection who tested positive to IgG anti-GOR did not differ from those who were GOR antibody-negative (table 1); the histological activity (average scores of necro-inflammation and fibrosis) tended to be greater, although not significantly, among IgG anti-GOR-positive patients with occult HCV infection (data not shown). On the other hand, the percentage of infected hepatocytes (that is, cells positive to genomic HCV RNA by in situ hybridization) resulted significantly greater ($P = 0.042$) in patients with occult HCV infection who tested positive to IgG anti-GOR (figure 3). However, the percentage of HCV-infected hepatocytes did not correlate significantly with IgG anti-GOR titres among the twenty-two GOR antibody-positive patients ($r_s = 0.311$, $P = 0.19$). In patients with overt chronic HCV infection the median percentage of infected hepatocytes observed by in situ hybridization was 8.0 (range 2.5 – 38.6), which resulted significantly higher ($P<0.001$) compared with occult HCV infection (median of 4.0, range 0.1 – 18.0), in agreement with a previous report (19).

With respect to rheumatoid factor, it was detected in the serum from 12 of the 110 (10.9%) patients with occult HCV infection, including one (4.5%) of the 22 GOR antibody-positive individuals. Similarly, C-reactive protein was detectable in 15/110 (13.6%) patients with occult HCV infection, including 1/22 (4.5%) IgG anti-GOR-positive individuals. Finally, cryoglobulins were found in 14/110 (12.7%) patients with occult HCV infection; only one of them (4.5%) had IgG anti-GOR detectable.

## DISCUSSION

In this study, we have observed a 20% frequency of IgG antibody reactivity to the GOR autoepitope in the serum of anti-HCV-negative patients with occult HCV infection. Low IgG anti-GOR titres were found in most GOR antibody-positive individuals. Importantly, IgG anti-GOR was not detected in any of the patients without HCV irrespective of the etiology of the liver disease. Thus, despite repeated absence of serum anti-HCV antibodies by commercial immunoassays IgG anti-GOR can be found in patients with occult HCV infection. Most studies had only detected anti-GOR reactivity in HCV seropositive patients (6,9,14,15,21,31). However, a few reports identified anti-HCV-negative individuals who tested positive to anti-GOR (9,14) including blood donors (7,32); although, these studies did not exclude the presence of occult HCV infection. In addition, it has been reported that detection of anti-GOR without anti-HCV is not associated with hepatitis C viremia (1). In this way, among occult HCV-infected patients HCV RNA is persistently negative in serum (2).

The frequency of IgG anti-GOR in occult HCV infection was significantly lower compared with a 63.6% GOR IgG-antibody reactivity found in patients with chronic hepatitis C, which is similar to the frequency reported by several authors in patients with overt HCV infection (10,12,14,16,21). Also, anti-GOR levels were greater in chronic hepatitis C compared with occult HCV infection. We have reported recently that sera from some patients with occult HCV infection may demonstrate a positive reaction against HCV non-structural proteins on immunoblot assays suggesting a very low level of specific antibody production (23). In chronic hepatitis C, the presence of antibodies reactive to the host-derived antigen GOR is not merely due to sequence homology but to cross-reactivity

at the molecular level because of conservation of residues essential for antibody binding (34). Thus, de novo infection with HCV after liver transplantation produces an increase in IgG anti-GOR likely due to increased viral load and replication under immunosuppresion indicating that the immune response to GOR autoantibody is triggered by HCV (24).

The low level of IgG anti-GOR antibodies detected in occult HCV infection may reflect not only exposure to HCV (22), but also an ongoing productive HCV infection within the liver (2). Indeed, HCV replication has been demonstrated in peripheral blood mononuclear cells from occult HCV-infected patients as well (3). This may result in discrete amounts of antigen production and then presentation to antibody-producing cells. Interestingly, the percentage of infected hepatocytes resulted significantly greater in patients with occult HCV infection who tested positive to IgG anti-GOR. The mechanism(s) that regulate humoral immune responses during occult HCV infection are not well known. In humans the GOR (GOR47-1) gene product cannot be translated into a protein (8) and so antibody responses to GOR and HCV may be independently regulated as suggested in chronic hepatitis C (11). In patients with chronic hepatitis C anti-HCV antibodies usually persist for decades; although, these may eventually disappear after recovery from HCV infection (29,30).

Among individuals with occult HCV infection, the subset of GOR IgG antibody-positive patients did not show a different clinical background compared with their IgG anti-GOR-negative counterparts (9). However, a greater number of IgG anti-GOR-positive patients had signs of necro-inflammation, which is similar to patients with chronic hepatitis C, in whom reactivity to GOR had been correlated with liver disease activity (21). Nevertheless, compared with chronic hepatitis C occult HCV infection seems to be a less

aggressive form of the disease caused by the hepatitis C virus (19); although, liver cirrhosis is present in around 4% of these patients.

Finally, rheumatoid factor, C-reactive protein and/or cryoglobulins were detected in the serum of 10-14% of occult HCV-infected patients. Frequencies of such factors were lower than those commonly found in chronic hepatitis (25), suggesting that this may reflect differences in the host response to HCV between occult HCV and chronic hepatitis C patients. In addition, the presence of these factors was not associated with the GOR IgG antibody status. These data are in line with the notion that the significance of GOR is little during triggering of autoimmune phenomena by HCV and thus GOR is unlikely a marker of induced autoimmunity as already reported in chronic HCV infection (13). Indeed, histological features of autoimmune disease were absent in all patients.

In conclusion, we have found that sera from 20% of the patients with occult HCV react with the GOR autoepitope on enzyme immunoassays; although, this frequency is lower compared to GOR reactivity in patients with chronic hepatitis C. Because IgG anti-GOR is not detected in patients with HCV-unrelated liver disease detection of IgG antibodies to the GOR seems to reflect cross-recognition with viral sequences during occult HCV infection, even in the absence of detectable HCV-specific antibodies using commercial tests. Testing for IgG anti-GOR might be used to screen HCV RNA-negative patients and thus help in identifying at least a subset of occult HCV infection without performing a liver biopsy. Nevertheless, even after implementation of IgG anti-GOR testing the majority of patients would still need a liver biopsy for accurate diagnosis of occult HCV infection.

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2

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1 **FIGURE LEGENDS**

2

3 **Fig. 1.** Specificity of serum anti-GOR detection assay in occult HCV infection. GOR
4 antibody detection was blocked by pre-incubation with GOR peptide but not by an irrelevant
5 peptide. The results shown are mean percentages of anti-GOR blocking at each peptide
6 concentration plus the standard error of the mean from five GOR antibody-positive
7 individuals with occult HCV infection.

8

9 **Fig. 2.** Comparison of GOR antibody titres between occult HCV infection and chronic
10 hepatitis C. Box-plot representation of anti-GOR titres (expressed as end-point serum dilution)
11 measured in GOR antibody-positive patients with occult HCV infection (n = 22) and chronic
12 hepatitis C (n = 70).

13

14 **Fig. 3.** Relationship between hepatic HCV infection and GOR antibody responses in
15 occult HCV infection. Box-plot representation of the percentages of HCV-infected
16 hepatocytes in occult HCV-infected patients with negative (n = 87) and positive (n = 21) anti-
17 GOR detection tests in serum. Outliers are represented by single circles.

**TABLE 1.** Characteristics of the patients with occult HCV infection according to GOR antibody status.

| | Anti-GOR positive (n=22) | Anti-GOR negative (n=88) | P value |
|---|---|---|---|
| Age (yr.) * | 45.5 (39.9-51.1) | 45.1 (42.7-47.5) | 0.86 |
| Gender (M/F) | 19/3 | 64/24 | 0.18 |
| Duration of disease (yr.) *,$ | 6.2 (3.4-9.0) | 6.4 (3.6-9.2) | 0.56 |
| ALT (IU/l) * | 67 (51-84) | 68 (59-79) | 0.67 |
| AST (IU/l) * | 38 (29-47) | 41 (35-46) | 0.86 |
| GGTP (IU/l) * | 111 (73-148) | 94 (76-112) | 0.32 |
| Necroinflammation ** | 11 (50) | 31 (35) | 0.20 |
| Fibrosis ** | 5 (23) | 17 (19) | 0.72 |
| Cirrhosis ** | 1 (4) | 2 (2) | 0.88 |
| Steatosis ** | 4 (18) | 14 (16) | 0.79 |





** expressed as the mean (95% CI of the mean).*

*** expressed as the number of cases (%).*

*$ refers to the estimated duration of abnormal liver function tests since first alteration was detected.*

**Figure 1**

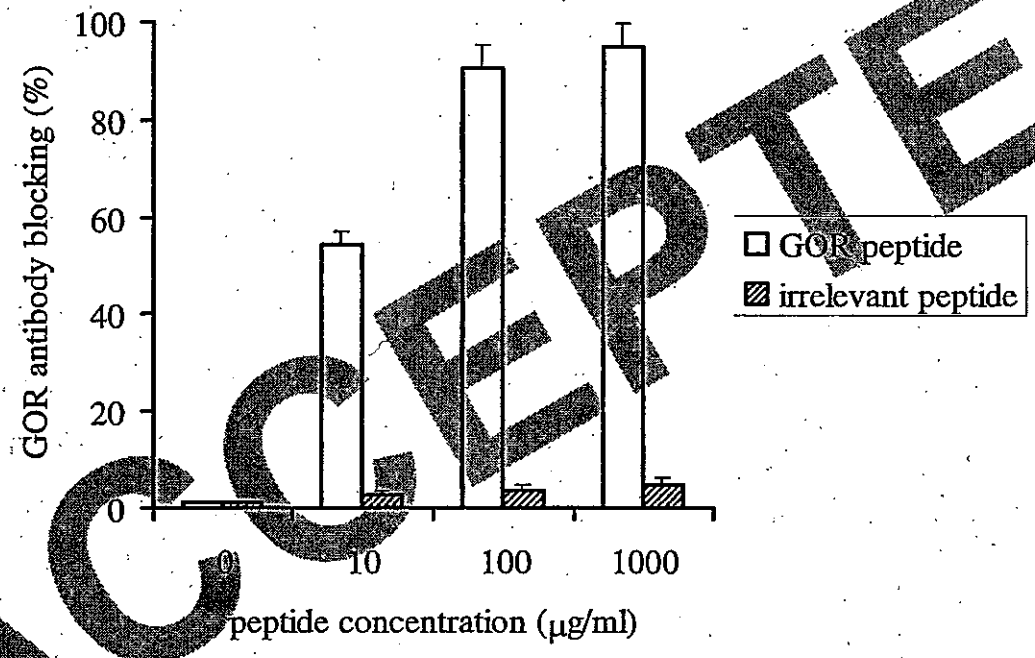


**Figure 2**

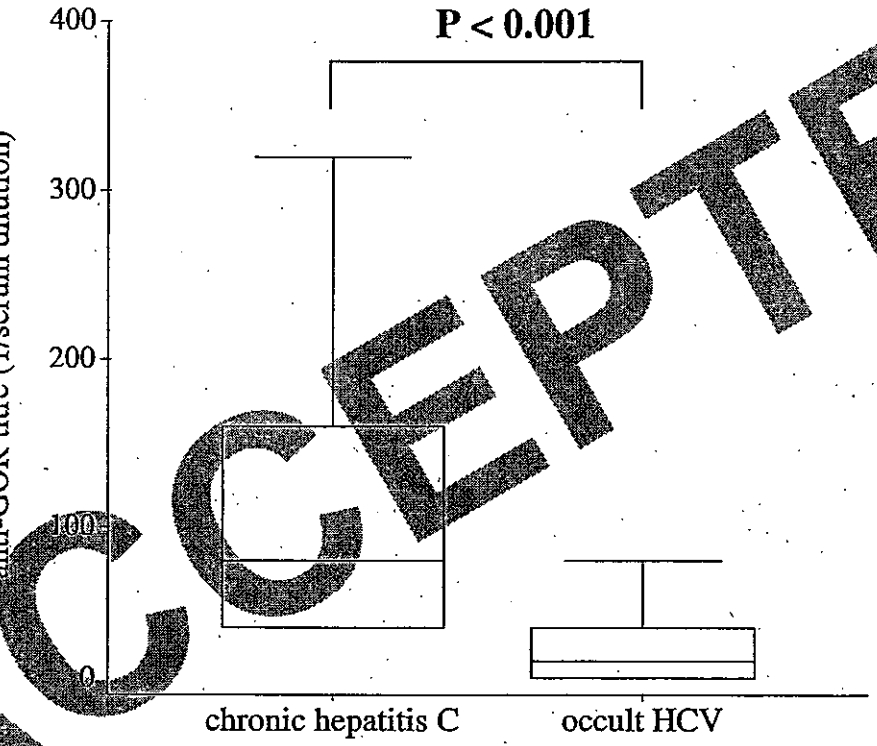
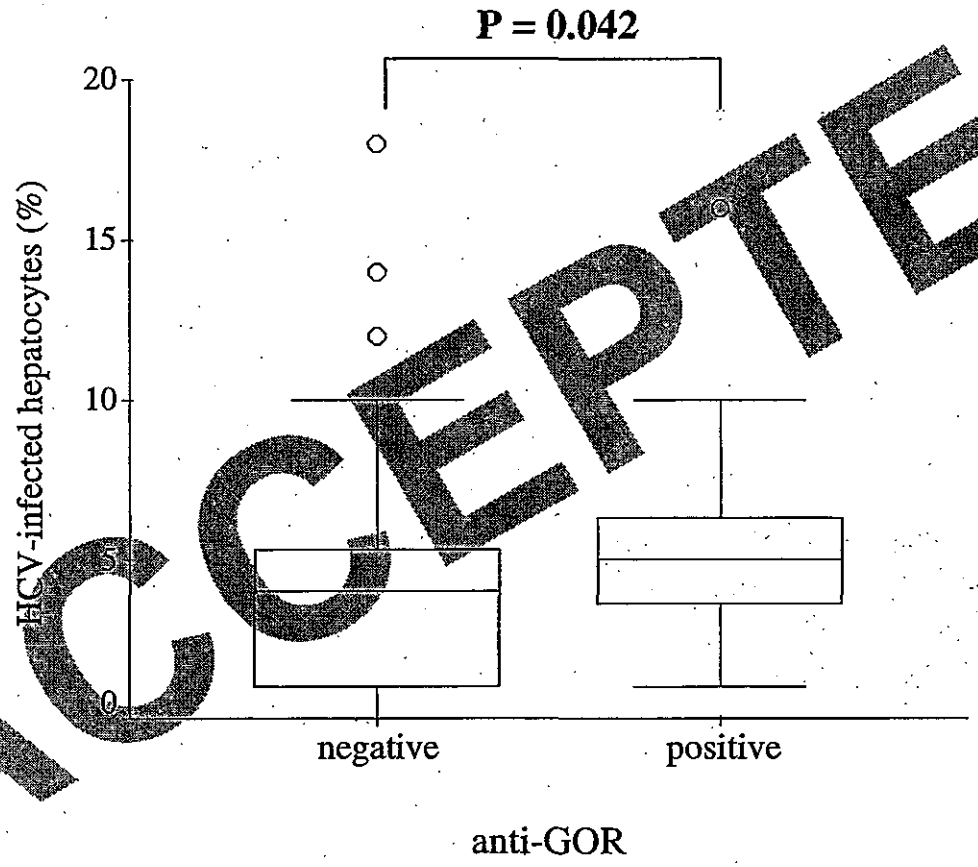


**Figure 3**



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**研究報告の概要**

○ALT上昇を伴う日本の献血者ではE型肝炎ウイルス抗体およびHEV RNAの高陽性率は1991年～2006年において変化していない

E型肝炎は本邦では稀であるが、今まで考えられていた以上に発生している。本邦において、E型肝炎ウイルス(HEV)の新たな不顕性感染症が近年増加しているかどうかを検討するため、1991年～2006年の期間に、ALT高値(≥61 IU/L)が認められ、現在HEVに感染している可能性の高い献血者4,019名から得た血清検体中のHEV RNAを調べた。2004年～2006年の献血者3,185名のanti-HEV IgG、anti-HEV IgM/IgA、HEV RNAの全体的な陽性率は、1998年の献血者594名と同等であった(5.3 vs. 5.2%、0.2 vs. 0.5%、0.2 vs. 0.3%)。献血年別に3群(1991年～1995年[n=156]、1996年～1999年[n=116]、2004年～2006年[n=61]に分けたALT ≥ 201 IU/Lの献血者間において、anti-HEV IgG(5.8、4.3、6.6%)、anti-HEV IgM/IgA(1.9、3.4、3.3%)、HEV RNA(1.3、3.4、3.3%)の陽性率に検出可能な差はなかった。本試験で得られた11のHEV分離ウイルスは、ORF2配列にそれぞれ1.7～22.8%の相違があり、遺伝子型3または4に分類された。1991年～2006年の期間に、本邦における多様なHEV株によるHEV不顕性感染の発現率は本質的に変化していない。

**使用上の注意記載状況・その他参考事項等**

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 日本において、1991年～2006年の期間にALT高値(≥61 IU/L)が認められ、現在HEVに感染している可能性が高い献血者の血清中のHEV RNAを調べたところ、この期間のHEV不顕性感染の発現率は本質的に変化していなかったとの報告である。 | 日本赤十字社では、厚生労働科学研究「E型肝炎の感染経路・宿主域・遺伝的多様性・感染防止・診断・治療に関する研究班」と共同して、献血者におけるHEV感染の疫学調査を行っている。北海道における輸血HEV感染報告を受け、試験的に北海道では研究的NATを行うなど安全対策を実施している。また、輸血による肝炎ウイルス感染防止のため、血液中のALT値61IU/L以上の血液を輸血用から排除している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。 |

(3)

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# Unchanged high prevalence of antibodies to hepatitis E virus (HEV) and HEV RNA among blood donors with an elevated alanine aminotransferase level in Japan during 1991–2006*

**S. Fukuda[1], M. Ishikawa[1], N. Ochiai[1], Y. Suzuki[1], J. Sunaga[1], N. Shinohara[1], K. Nozawa[1], F. Tsuda[2], M. Takahashi[3], H. Okamoto[3]**

[1] Japanese Red Cross Tochigi Blood Center, Tochigi, Japan
[2] Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan
[3] Division of Virology, Department of Infection and Immunity, Jichi Medical University School of Medicine, Tochigi, Japan

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## Summary

Hepatitis E is rare in Japan but is occurring more frequently than previously thought. To investigate whether de novo subclinical infection of hepatitis E virus (HEV) has recently increased in Japan, HEV RNA was assayed in serum samples obtained from 4019 Japanese voluntary blood donors with alanine aminotransferase (ALT) of ≥61 IU/l, who are likely to have ongoing HEV infection, during 1991–2006. The overall rates of IgG-class antibody to HEV (anti-HEV IgG), anti-HEV IgM/IgA and HEV RNA among 3185 donors in 2004–2006 were comparable with those among 594 donors in 1998 (5.3 vs. 5.2%, 0.2 vs. 0.5%, and 0.2 vs. 0.3%, respectively). Among blood donors with ALT ≥ 201 IU/l in three groups according to the year of blood collection (1991–1995 [$n=156$], 1996–1999 [$n=116$] and 2004–2006 [$n=61$]), there were no appreciable differences in the prevalence of anti-HEV IgG (5.8, 4.3, and 6.6%, respectively), anti-HEV IgM/IgA (1.9, 3.4, and 3.3%, respectively) and HEV RNA (1.3, 3.4, and 3.3%, respectively). The eleven HEV isolates obtained in the present study differed from each other by 1.7–22.8% in the ORF2 sequence and segregated into genotype 3 or 4. The occurrence rate of subclinical infection with divergent HEV strains has essentially remained unchanged during 1991–2006 in Japan.

## Introduction

Hepatitis E, which is caused by hepatitis E virus (HEV), is found in many parts of the world. The disease is transmitted via the fecal-oral route through virus-contaminated water or food in developing countries where sanitation is suboptimal [36]. HEV infection is also endemic in industrialized countries, and IgG-class antibodies against HEV (anti-HEV IgG), most likely due to past subclinical HEV

* The nucleotide sequence data reported in this study have been assigned DDBJ/EMBL/GenBank accession numbers AB292649–AB292659.

Correspondence: Hiroaki Okamoto, Division of Virology, Department of Infection and Immunity, Jichi Medical University School of Medicine, 3311-1 Yakushiji, Shimotsuke-Shi, Tochigi-Ken 329-0498, Japan
e-mail: hokamoto@jichi.ac.jp

infection, have been detected in a significant proportion of healthy individuals in the United States, European countries and Japan [8, 16, 33, 36, 40, 51]; however, only a limited number of sporadic cases of acute hepatitis E have been reported in industrialized countries. Increasing lines of evidence indicate that hepatitis E is a zoonosis and that there exist animal reservoirs of HEV [9, 24, 25, 31–33, 40, 48, 52, 57].

HEV is a single-stranded, positive-sense RNA virus without an envelope and is currently classified as the sole member of the genus *Hepevirus* in the family *Hepeviridae* [4]. Its genome is approximately 7.2 kilobases (kb) in length and contains three open reading frames (ORFs: ORF1, ORF2, and ORF3) flanked by short untranslated regions [49]. ORF1 encodes non-structural proteins that are involved in virus replication and viral protein processing. ORF2 encodes the capsid protein and ORF3 encodes a small phosphorylated protein [14, 58]. Due to the extensive genomic diversity noted among HEV isolates, HEV sequences have been classified into four genotypes (genotypes 1–4) [39, 56]. Genotype 1 HEV was responsible for a number of waterborne epidemics of hepatitis E in Asia and Africa. Although HEV of genotype 2 has been detected less frequently, it was responsible for outbreaks in Mexico in 1986–1987 [54] and has been implicated in sporadic infections in Africa [3, 20]. On the other hand, genotypes 3 and 4 HEV cause sporadic cases of acute hepatitis but have not been found to be responsible for epidemics in humans; these infections seem to be zoonotic and both genotypes have been detected in pigs (genotype 3 worldwide, and genotype 4 in East Asia), which may constitute the major reservoir of HEV genotypes 3 and 4 [8, 19, 24, 25].

In Japan, multiple HEV strains of genotypes 3 and 4 have been recovered from patients with domestically acquired hepatitis E [12, 30, 42–45], and HEV has been recognized as an important causative agent of sporadic acute hepatitis of non-A, non-B, non-C etiology [30, 33]. A high prevalence of anti-HEV IgG has been reported [16, 27, 28, 50, 51], and HEV-viremic subjects have been identified among symptom-free blood donors with an elevated alanine aminotransferase (ALT) level [7]. However, it remains unknown whether or not subclinical HEV infection is increasing recently in Japan. Therefore, in an attempt to investigate the changing prevalence of de novo subclinical HEV infection in Japan, HEV RNA was assayed in serum samples obtained from Japanese voluntary blood donors with an elevated ALT level of $\geq$61 IU/l, who are likely to have ongoing HEV infection, during 1991–2006.

## Materials and methods

### *Serum samples*

Serum samples were collected from a total of 3185 voluntary blood donors (age: 32.5 ± 10.9 [mean ± standard deviation, SD] years; 2863 men and 322 women) with an elevated ALT level of 61–967 (range: 87.9 ± 41.8, mean ± SD) IU/l at the Japanese Red Cross Tochigi Blood Center, Japan, between April 2004 and December 2006. The Blood Center is located in Tochigi Prefecture, a prefecture in the northern part of mainland Honshu of Japan. Serum samples collected from 594 blood donors with an elevated ALT level of 61–2178 (100.4 ± 106.9) IU/l between February and November 1998 at the same blood center were also used in the present study. In addition, serum samples obtained from 240 blood donors with an elevated ALT level of $\geq$201 IU/l at the same blood center, from 1991–1997 and 1999, were used. Serum samples obtained from repeat donors during the study period were excluded; that is, each sample was obtained from a unique individual.

All 4019 serum samples were negative for hepatitis B surface antigen, and antibodies to hepatitis C virus (HCV) and human immunodeficiency virus (HIV) type 1. The 3198 samples obtained since 1999 were additionally negative for hepatitis B virus DNA, HCV RNA and HIV type 1 RNA by the nucleic acid amplification test using Roche's Multiplex reagent [26].

### *Detection of antibodies to HEV*

To detect anti-HEV IgG, anti-HEV IgM and anti-HEV IgA, enzyme-linked immunosorbent assay (ELISA) was performed using purified recombinant ORF2 protein of HEV genotype 4 that had been expressed in the pupae of silkworm [30], as described previously [47]. In the ELISA assays for anti-HEV IgG, anti-HEV IgM and anti-HEV IgA, peroxidase-conjugated mouse monoclonal anti-human IgG antibody, peroxidase-conjugated mouse monoclonal anti-human IgM, or peroxidase-labeled mouse monoclonal anti-human IgA, respectively, was used. The optical density (OD) of each sample was read at 450 nm. The cut-off value used for the anti-HEV IgG, anti-HEV IgM, and anti-HEV IgA assays was 0.175, 0.440, and 0.642, respectively [47]. Samples with OD values for anti-HEV IgG, IgM, or IgA equal to or greater

than the respective cut-off value were considered to be positive for anti-HEV IgG, IgM, or IgA, respectively. The specificity of the anti-HEV assays was verified by absorption with the same recombinant ORF2 protein that was used as the antigen probe. Briefly, when the OD value of the tested sample was less than 30% (anti-HEV IgG/IgA) or 50% (anti-HEV IgM) of the original value after absorption with the recombinant ORF2 protein, the sample was considered to be positive for anti-HEV.

*Detection of HEV RNA*

Reverse transcription (RT)-polymerase chain reaction (PCR) was performed for detection of HEV RNA in serum samples with anti-HEV IgM and/or anti-HEV IgA, using nested primers targeting the ORF2 region, as described previously [30]. The size of the amplification product of the first-round PCR was 506 base pairs (bp), and that of the second-round PCR was 457 bp. The nested RT-PCR assay that we used has the capability of amplifying all four known genotypes of HEV strains reported thus far [30, 46, 57]. The specificity of the RT-PCR assay was verified by sequence analysis as described below. The sensitivity of the RT-PCR assay was assessed as described previously [30]. For serum samples that were negative for HEV RNA when 100 µl of serum sample was used, total RNA was extracted from 500 µl of serum, reverse transcribed, and then subjected to nested PCR as described above. To extract RNA from 500 µl of serum, test serum diluted 2-fold in saline was centrifuged at 287,582 × *g* at 4 °C for 2 h in a TLA-100.2 rotor (Beckman Coulter K. K., Tokyo, Japan), and the resulting pellet was suspended in 100 µl of saline and subjected to the RT-PCR assay. To confirm the reproducibility, this assay was performed in duplicate.

For serum samples without anti-HEV IgM and anti-HEV IgA, 10 µl each from 50 serum samples were pooled, and each pool was tested for HEV RNA by the above-mentioned RT-PCR. If a pool was positive for HEV RNA, the 50 serum samples of that pool were individually tested for the presence of HEV RNA.

*Sequence analysis of PCR products*

The amplification products were sequenced directly on both strands using the BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence analysis was performed using Genetyx-Mac version 12.2.7 (Genetyx Corp., Tokyo, Japan) and ODEN version 1.1.1 from the DNA Data Bank of Japan (DDBJ: National Institute of Genetics, Mishima, Japan) [11]. Sequence alignments were generated by CLUSTAL W (version 1.8) [53]. Phylogenetic trees were constructed by the neighbor-joining method [38] based on the partial nucleotide sequence of the ORF2 region (412 nucleotides [nt]). Bootstrap values were determined on 1000 resamplings of the data sets [6].

*Statistical analysis*

Statistical analyses were performed using the Chi-Square-test for comparison of proportions between two groups. Differences were considered to be statistically significant at $P<0.05$.

## Results

### *Age- and sex-specific prevalence of anti-HEV antibodies and HEV RNA during 2004–2006*

A total of 3185 serum samples obtained from apparently healthy blood donors with an elevated ALT level between April 2004 and December 2006 were tested for the presence of anti-HEV IgG. Anti-HEV IgG was detected in 5.3% (168/3185) of the tested population including 5.1% of the 2863 male donors and 6.5% of the 322 female donors, the difference not being significant (Table 1). The prevalence of anti-HEV IgG increased with age among both the male and female donors, and was significantly higher among donors aged ≥40 years than among those aged <40 years in total (10.9 vs. 3.4%, $P<0.0001$) and in the males (11.0 vs. 3.2%, $P<0.0001$). All 168 serum samples with anti-HEV IgG were tested for anti-HEV IgM and anti-HEV IgA. Among them, anti-HEV IgM and anti-HEV IgA were simultaneously detected in six samples (3.6%), of which four samples tested positive for HEV RNA in a sample volume of both 10 and 100 µl, and one sample in 500 µl. As for the 3179 serum samples without anti-HEV IgM and anti-HEV IgA, although sixty-three 50-sample pools and one 29-sample pool were tested for the presence of HEV RNA, none of them had detectable HEV RNA. Consequently, 5 (0.2%) of the 3185 samples were found to be viremic for HEV. As for the prevalence of HEV viremia, there were no appreciable differences between males and females (0.1 vs. 0.3%, $P=0.4628$), and between donors aged ≥40 years and those aged <40 years (0.4 vs. 0.1%, $P=0.0700$).

### *Prevalence of anti-HEV antibodies and HEV RNA during 2004–2006, stratified by ALT level*

In the present study, 168 donors with anti-HEV IgG were found during 2004–2006, including 143 (5.6%) with an ALT level of 61–100 IU/l, 21 (3.6%)

**Table 1.** Age- and sex-dependent prevalence of anti-HEV antibodies and HEV RNA among voluntary blood donors with an elevated ALT level between April 2004 and December 2006

| Age (years) | No. of total donors (%) with | | | | No. of male donors (%) with | | | | No. of female donors (%) with | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of donors | Anti-HEV | | HEV RNA | No. of donors | Anti-HEV | | HEV RNA | No. of donors | Anti-HEV | | HEV RNA |
| | | IgG-class | IgM- and/or IgA-class | | | IgG-class | IgM- and/or IgA-class | | | IgG-class | IgM- and/or IgA-class | |
| 16–19 | 538 | 9 (1.7) | 1 (0.2) | 1 (0.2) | 462 | 7 (1.5) | 1 (0.2) | 1 (0.2) | 76 | 2 (2.6) | 0 | 0 |
| 20–29 | 702 | 19 (2.7) | 1 (0.1) | 1 (0.1) | 640 | 14 (2.2) | 1 (0.2) | 1 (0.2) | 62 | 5 (8.1) | 0 | 0 |
| 30–39 | 1150 | 53 (4.6) | 0 | 0 | 1054 | 48 (4.6) | 0 | 0 | 96 | 5 (5.2) | 0 | 0 |
| 40–49 | 561 | 55 (9.8) | 2 (0.4) | 1 (0.2) | 518 | 54 (10.4) | 2 (0.4) | 1 (0.2) | 43 | 1 (2.3) | 0 | 0 |
| 50–59 | 200 | 24 (12.0) | 0 | 0 | 169 | 19 (11.2) | 0 | 0 | 31 | 5 (16.1) | 0 | 0 |
| 60–68 | 34 | 8 (23.5) | 2 (5.9) | 2 (5.9) | 20 | 5 (25.0) | 1 (5.0) | 1 (5.0) | 14 | 3 (21.4) | 1 (7.1) | 1 (7.1) |
| Total | 3185 | 168 (5.3) | 6 (0.2) | 5 (0.2) | 2863 | 147 (5.1) | 5 (0.2) | 4 (0.1) | 322 | 21 (6.5) | 1 (0.3) | 1 (0.3) |

with an ALT level of 101–200 IU/l, and 4 (6.6%) with an ALT level of ≥201 IU/l (Table 2). The prevalence of anti-HEV IgG was comparable between donors with an ALT level of ≥201 IU/l and those with an ALT level of 61–200 IU/l (6.6 vs. 5.2%). As for the prevalence of HEV RNA, however, there was a significant difference between donors with an ALT level of ≥201 IU/l and those with an ALT level of 61–200 IU/l in total (3.3 vs. 0.1%, $P<0.0001$), in males (3.7 vs. 0.1%, $P<0.0001$), but not in females, probably due to the small number of female donors tested (0 vs. 0.3%, $P=0.8813$).

*Comparison of the prevalence of anti-HEV antibodies and HEV RNA between donors with an elevated ALT level in 1998 and those in 2004–2006*

The overall rates of anti-HEV IgG, anti-HEV IgM/IgA and HEV RNA among donors in 1998 were comparable with those among donors in 2004–2006 (5.2 vs. 5.3%, 0.5 vs. 0.2%, and 0.3 vs. 0.2%, respectively) (Table 3). The prevalence of anti-HEV IgG increased with age in the two year groups, although none of the two donors in the age group of 60–68 years in 1998 had anti-HEV IgG. As in the year group of 2004–2006, the prevalence of HEV RNA was significantly higher among donors with an ALT level of ≥201 IU/l than among those with an ALT level of 61–200 IU/l in 1998 (6.3 vs. 0%, $P<0.0001$).

*Prevalence of anti-HEV antibodies and HEV RNA among donors with an elevated ALT level of ≥201 IU/l, stratified by the year group of blood collection*

Table 4 compares various features of the blood donors with an elevated ALT level of ≥201 IU/l, who are likely to have ongoing HEV infection, in the three year groups (1991–1995, 1996–1999 and 2004–2006) according to the year of blood collection. There were no appreciable differences in the age distribution, gender ratio, ALT level and prevalence of anti-HEV IgG among the three year groups of 1991–1995, 1996–1999 and 2004–2006. The prevalence of anti-HEV IgM/IgA and HEV RNA, indicative of present HEV infection, was low at 1.9

**Table 2.** Prevalence of anti-HEV IgG and HEV RNA among voluntary blood donors with an elevated ALT level during 2004–2006, stratified by ALT level

| ALT (IU/l) | Total | | | Male | | | Female | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | Anti-HEV IgG | HEV RNA | N | Anti-HEV IgG | HEV RNA | N | Anti-HEV IgG | HEV RNA |
| 61–100 | 2546 | 143 (5.6%) | 2 (0.1%) | 2296 | 128 (5.6%) | 1 (0.04%) | 250 | 15 (6.0%) | 1 (0.4%) |
| 101–200 | 578 | 21 (3.6%) | 1 (0.2%) | 513 | 15 (2.9%) | 1 (0.2%) | 65 | 6 (9.2%) | 0 |
| 201–967 | 61 | 4 (6.6%) | 2 (3.3%) | 54 | 4 (7.4%) | 2 (3.7%) | 7 | 0 | 0 |
| Total | 3185 | 168 (5.3%) | 5 (0.2%) | 2863 | 147 (5.1%) | 4 (0.1%) | 322 | 21 (6.5%) | 1 (0.3%) |

**Table 3.** Comparison of the prevalence of anti-HEV antibodies and HEV RNA among blood donors with an elevated ALT level between 1998 and 2004–2006

| Feature | 1998 | | | | 2004–2006 | | | |
|---|---|---|---|---|---|---|---|---|
| | N | Anti-HEV-positive (%) | | HEV RNA-positive (%) | N | Anti-HEV-positive (%) | | HEV RNA-positive (%) |
| | | IgG-class | IgM- and/or IgA-class | | | IgG-class | IgM- and/or IgA-class | |
| Age (years) | | | | | | | | |
| 16–19 | 43 | 0 | 0 | 0 | 538 | 9 (1.7) | 1 (0.2) | 1 (0.2) |
| 20–29 | 216 | 6 (2.8) | 1 (0.5) | 1 (0.5) | 702 | 19 (2.7) | 1 (0.1) | 1 (0.1) |
| 30–39 | 200 | 11 (5.5) | 0 | 0 | 1150 | 53 (4.6) | 0 | 0 |
| 40–49 | 108 | 10 (9.3) | 1 (0.9) | 0 | 561 | 55 (9.8) | 2 (0.4) | 1 (0.2) |
| 50–59 | 25 | 4 (16.0) | 1 (4.0) | 1 (4.0) | 200 | 24 (12.0) | 0 | 0 |
| 60–68 | 2 | 0 | 0 | 0 | 34 | 8 (23.5) | 2 (5.9) | 2 (5.9) |
| ALT (IU/l) | | | | | | | | |
| 61–100 | 454 | 23 (5.1) | 1 (0.2) | 0 | 2546 | 143 (5.6) | 3 (0.1) | 2 (0.1) |
| 101–200 | 108 | 7 (6.5) | 0 | 0 | 578 | 21 (3.6) | 1 (0.2) | 1 (0.2) |
| 201–2178 | 32 | 1 (3.1) | 2 (6.3) | 2 (6.3) | 61 | 4 (6.6) | 2 (3.3) | 2 (3.3) |
| Total | 594 | 31 (5.2) | 3 (0.5) | 2 (0.3) | 3185 | 168 (5.3) | 6 (0.2) | 5 (0.2) |

**Table 4.** Prevalence of anti-HEV antibodies and HEV RNA among blood donors with an elevated ALT level of ≥201 IU/l, stratified by the year group of blood collection

| Feature | Year of blood collection | | |
|---|---|---|---|
| | 1991–1995 ($n=156$) | 1996–1999 ($n=116$) | 2004–2006 ($n=61$) |
| Age (mean ± SD, years) | 26.0 ± 9.9 | 26.4 ± 9.9 | 27.6 ± 12.5 |
| Male | 145 (92.9%) | 105 (90.5%) | 54 (88.5%) |
| ALT (mean ± SD, IU/l) | 294.6 ± 216.4 | 299.8 ± 211.6 | 289.4 ± 135.8 |
| Anti-HEV IgG | 9 (5.8%) | 5 (4.3%) | 4 (6.6%) |
| Anti-HEV IgM/IgA | 3 (1.9%) | 4 (3.4%) | 2 (3.3%) |
| HEV RNA | 2 (1.3%)[a,b] | 4 (3.4%)[a,c] | 2 (3.3%)[b,c] |
| HEV genotype | | | |
| Genotype 3 | 1 (50.0%) | 2 (50.0%) | 2 (100%) |
| Genotype 4 | 1 (50.0%) | 2 (50.0%) | 0 |

[a] $P=0.2290$.
[b] $P=0.3256$.
[c] $P=0.9528$.

and 1.3%, respectively, in the year group of 1991–1995, but the difference among the three year groups was not statistically significant.

*Genetic analysis of HEV isolates recovered from 11 viremic donors*

The 11 HEV isolates recovered from the transiently viremic donors were named with the prefix of HE-JTB followed by the year of isolation and the sequential number of the viremic samples obtained that year (Table 5). The 412-nt sequence of ORF2 of these HEV isolates was determined and compared with each other and with that of known HEV isolates of genotypes 1–4. These 11 HEV isolates were markedly variable, sharing nucleotide identities ranging from 77.2 to 98.3%, and were classifiable into two groups differing by 18.9–22.8%. Eight HEV isolates (HE-JTB95-1, HE-JTB96-1, HE-JTB96-2, HE-JTB05-1, HE-JTB05-2, HE-JTB06-1, HE-JTB06-2, and HE-JTB06-3) comprised one group and were close to the prototype Japanese isolate of genotype 3 (JRA1 [accession no. AP003430]) with nucleotide identities of 85.9–93.0%, and were only 77.9–80.3, 74.8–77.4, and 77.9–80.1% similar to the B1 isolate (M73218) of genotype 1, MEX-14 isolate (M74506) of genotype 2, and T1 isolate (AJ272108) of genotype 4, respectively. This finding suggests that these 8 HEV isolates are classifiable into genotype 3. The phylogenetic tree constructed based on the common 412-nt sequence within the ORF2 sequence confirmed that these 8 HEV isolates obtained in the present study belonged to genotype 3 and showed that they segregated into clusters consisting of Japanese HEV strains of the same genotype that had been recovered from hu-

**Table 5.** Characteristics of blood donors with an elevated ALT level who had detectable HEV RNA

| Year of isolation | Age (years)/sex | ALT (IU/l) | Anti-HEV ($OD_{450}$ value) | | | HEV RNA (μl)[a] | | | HEV genotype | Isolate name |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | IgG-class | IgM-class | IgA-class | 10 | 100 | 500 | | |
| 1994 | 44/M | 457 | >3.000 (+) | 2.325 (+) | 2.137 (+) | +[b] | + | NT[c] | 4 | HE-JTB94-1 |
| 1995 | 58/M | 2598 | 0.415 (+) | 0.611 (+) | 1.171 (+) | + | + | NT | 3 | HE-JTB95-1 |
| 1996 | 47/F | 215 | 1.629 (+) | 1.900 (+) | >3.000 (+) | + | + | NT | 3 | HE-JTB96-1 |
| 1996 | 49/M | 262 | 1.624 (+) | 0.981 (+) | 1.983 (+) | + | + | NT | 3 | HE-JTB96-2 |
| 1998 | 29/M | 628 | 0.127 (−) | 1.272 (+) | 0.146 (−) | + | + | NT | 4 | HE-JTB98-1 |
| 1998 | 54/M | 2178 | 0.439 (+) | 1.030 (+) | 1.265 (+) | + | + | NT | 4 | HE-JTB98-2 |
| 2005 | 61/M | 967 | 1.762 (+) | 1.967 (+) | 2.825 (+) | + | + | NT | 3 | HE-JTB05-1 |
| 2005 | 25/M | 85 | >3.000 (+) | 2.792 (+) | 2.683 (+) | + | + | NT | 3 | HE-JTB05-2 |
| 2006 | 60/F | 63 | 1.170 (+) | 0.665 (+) | 2.928 (+) | + | + | NT | 3 | HE-JTB06-1 |
| 2006 | 17/M | 138 | 2.278 (+) | 1.804 (+) | 2.939 (+) | − | − | + | 3 | HE-JTB06-2 |
| 2006 | 49/M | 758 | 2.866 (+) | >3.000 (+) | 2.313 (+) | + | + | NT | 3 | HE-JTB06-3 |

[a] HEV RNA was assayed using the indicated volume of serum samples.
[b] +, positive for HEV RNA; −, negative for HEV RNA.
[c] NT, not tested.

**Fig. 1.** Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence of the ORF2 region (412 nt) of 99 HEV isolates, using a genotype 1 HEV (M73218) as an outgroup. In addition to the HE-JTB95-1, HE-JTB96-1, HE-JTB96-2, HE-JTB05-1, HE-JTB05-2, HE-JTB06-1, HE-JTB06-2, and HE-JTB06-3 isolates found in the present study, which are indicated in bold type for visual clarity, 90 reported HEV isolates of genotype 3, whose common 412-nt sequence is known, are included for comparison. The reported isolates are indicated with the accession no. followed by the name of the country of isolation (non-Japanese origin only). An asterisk denotes human or swine HEV strains that were isolated in the same prefecture as those obtained in the present study. Bootstrap values are indicated for the major nodes as a percentage obtained from 1000 resamplings of the data

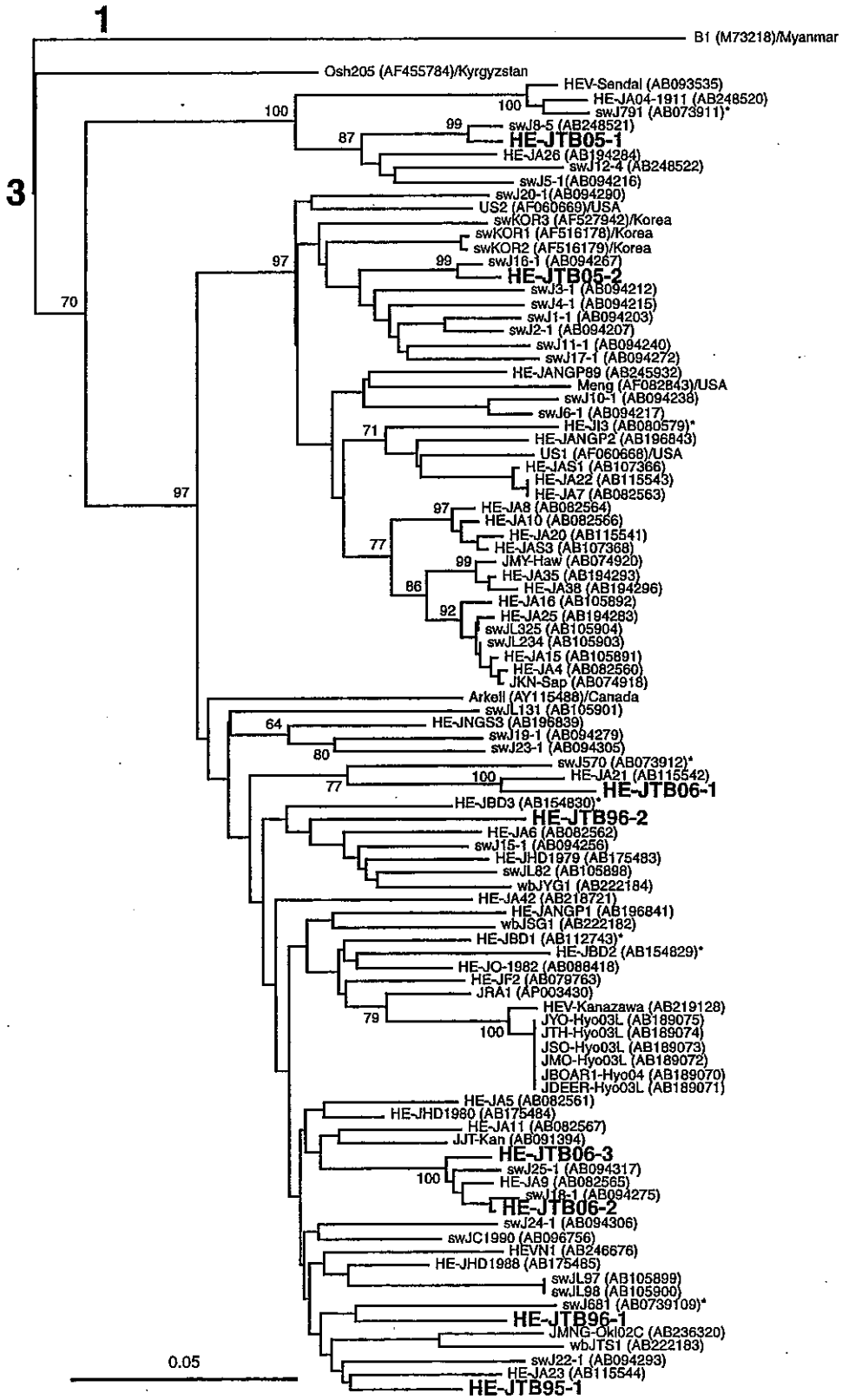
1
3
B1 (M73218)/Myanmar
Osh205 (AF455784)/Kyrgyzstan
HEV-Sendai (AB093535)
HE-JA04-1911 (AB248520)
swJ791 (AB073911)*
swJ8-5 (AB248521)
HE-JTB05-1
HE-JA26 (AB194284)
swJ12-4 (AB248522)
swJ5-1(AB094216)
swJ20-1(AB094290)
US2 (AF060669)/USA
swKOR3 (AF527942)/Korea
swKOR1 (AF516178)/Korea
swKOR2 (AF516179)/Korea
swJ16-1 (AB094267)
HE-JTB05-2
swJ3-1 (AB094212)
swJ4-1 (AB094215)
swJ1-1 (AB094203)
swJ2-1 (AB094207)
swJ11-1 (AB094240)
swJ17-1 (AB094272)
HE-JANGP89 (AB245932)
Meng (AF082843)/USA
swJ10-1 (AB094238)
swJ6-1 (AB094217)
HE-JI3 (AB080579)*
HE-JANGP2 (AB196843)
US1 (AF060668)/USA
HE-JAS1 (AB107366)
HE-JA22 (AB115543)
HE-JA7 (AB082563)
HE-JA8 (AB082564)
HE-JA10 (AB082566)
HE-JA20 (AB115541)
HE-JAS3 (AB107368)
JMY-Haw (AB074920)
HE-JA35 (AB194293)
HE-JA38 (AB194296)
HE-JA16 (AB105892)
HE-JA25 (AB194283)
swJL325 (AB105904)
swJL234 (AB105903)
HE-JA15 (AB105891)
HE-JA4 (AB082560)
JKN-Sap (AB074918)
Arkell (AY115488)/Canada
swJL131 (AB105901)
HE-JNGS3 (AB196839)
swJ19-1 (AB094279)
swJ23-1 (AB094305)
swJ570 (AB073912)*
HE-JA21 (AB115542)
HE-JTB06-1
HE-JBD3 (AB154830)*
HE-JTB96-2
HE-JA6 (AB082562)
swJ15-1 (AB094256)
HE-JHD1979 (AB175483)
swJL82 (AB105898)
wbJYG1 (AB222184)
HE-JA42 (AB218721)
HE-JANGP1 (AB196841)
wbJSG1 (AB222182)
HE-JBD1 (AB112743)*
HE-JBD2 (AB154829)*
HE-JO-1982 (AB088418)
HE-JF2 (AB079763)
JRA1 (AP003430)
HEV-Kanazawa (AB219128)
JYO-Hyo03L (AB189075)
JTH-Hyo03L (AB189074)
JSO-Hyo03L (AB189073)
JMO-Hyo03L (AB189072)
JBOAR1-Hyo04 (AB189070)
JDEER-Hyo03L (AB189071)
HE-JA5 (AB082561)
HE-JHD1980 (AB175484)
HE-JA11 (AB082567)
JJT-Kan (AB091394)
HE-JTB06-3
swJ25-1 (AB094317)
HE-JA9 (AB082565)
swJ18-1 (AB094275)
HE-JTB06-2
swJ24-1 (AB094306)
swJC1990 (AB096756)
HEVN1 (AB246676)
HE-JHD1988 (AB175485)
swJL97 (AB105899)
swJL98 (AB105900)
swJ681 (AB0739109)*
HE-JTB96-1
JMNG-Okl02C (AB236320)
wbJTS1 (AB222183)
swJ22-1 (AB094293)
HE-JA23 (AB115544)
HE-JTB95-1
0.05


**Fig. 2.** Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence of the ORF2 region (412 nt) of 65 HEV isolates, using a genotype 1 HEV (M73218) as an outgroup. In addition to the HE-JTB94-1, HE-JTB98-1, and HE-JTB98-2 isolates found in the present study, which are indicated in bold type, 61 reported HEV isolates of genotype 4, whose common 412-nt sequence is known, are included for comparison. The reported isolates are indicated with the accession no. followed by the name of the country where it was isolated (non-Japanese origin only). An asterisk denotes human HEV strains that were isolated in the same prefecture as those obtained in the present study. Bootstrap values are indicated for the major nodes as a percentage obtained from 1000 resamplings of the data

mans, swine and wild boars, supporting the indigenous nature of these 8 blood donor isolates (Fig. 1). On the other hand, the remaining three HEV isolates obtained in the present study (HE-JTB94-1, HE-JTB98-1 and HE-JTB98-2) were close to the prototype genotype 4 isolate (T1) with nu-

cleotide identities of 84.7–87.6%, and were only 79.9–81.8, 77.7–78.4, and 79.9–81.3% similar to the B1 isolate of genotype 1, MEX-14 isolate of genotype 2, and JRA1 isolate of genotype 3, respectively, suggesting that the HE-JTB94-1, HE-JTB98-1 and HE-JTB98-2 isolates belong to genotype 4. The phylogenetic tree constructed based on the common 412-nt ORF2 sequence confirmed that the 3 HEV isolates obtained in the present study segregated to genotype 4 (Fig. 2). Of note, the HE-JTB94-1 and HE-JTB98-1 isolates segregated into two distinct clusters consisting of Japanese HEV strains, each with a bootstrap value of 100%, but HE-JTB98-2 segregated into a cluster comprising the Chinese HEV strains that had been recovered from a Chinese patient with autochthonous hepatitis E (accession no. EF077630) and Japanese patients with hepatitis E who had traveled to China (AB197673–AB197674), suggesting that the HE-JTB98-2 isolate may be of China origin.

## Discussion

This study examined the prevalence of ongoing subclinical HEV infection among 4019 apparently healthy blood donors with an elevated ALT level of ≥61 IU/l who donated blood during the last 16 years at a Japanese Red Cross Blood Center located in the northern part of mainland Honshu of Japan. As for the geographical distribution of hepatitis E in Japan, it was reported that there was wide variation, with a higher prevalence in the northern part of Japan (Hokkaido Island and the northern part of mainland Honshu) [1, 30], suggesting that the results obtained in the present study cannot simply be generalized for the whole country. However, our study corroborated the previous study by Tanaka et al. [51], who reported that, based on the age-specific distribution of anti-HEV IgG in 1974, 1984, and 1994, exposure to HEV remained constant between 1974 and 1994 in Metropolitan Tokyo, Japan. The prevalence of clinical HEV infection among patients with acute hepatitis remained unchanged during the period from 1989 to 2005 in a city hospital in Aichi Prefecture, which is located in the central part of Honshu Island of Japan [29]. Therefore, our present study may represent the recent trends of HEV infection, at least in the northern and central parts of mainland Honshu of Japan, where hepatitis E is low-endemic [1].

The presence of anti-HEV IgG most likely reflects past subclinical HEV infection. The present study revealed that the prevalence of anti-HEV IgG among blood donors with ALT of ≥61 IU/l between 2004 and 2006 was similar to that in 1998 (5.3 vs. 5.2%), and that the prevalence of anti-HEV IgG among blood donors with ALT of ≥201 IU/l was comparable among the three year groups of 1991–1995, 1996–1999 and 2004–2006 (5.8, 4.3, and 6.6%, respectively) as well as that of 2002–2003 (4.1% or 23/560) [7]. Longitudinal seroepidemiological studies on transiently infected individuals suggested that anti-HEV IgG persisted much longer than expected, i.e., for more than 20 years [27, 28]. Even a low titer of anti-HEV IgG may reflect past subclinical HEV infection and has been detected in a significant proportion of healthy individuals not only in Japan but also in the United States and European countries [8, 16, 33, 36, 40, 51]. Therefore, in studies in which anti-HEV IgG is assayed at a single time point in each individual, it may be hard to specify when individuals with anti-HEV IgG contracted HEV infection and how prevalent de novo subclinical HEV infection was during a particular period.

In the present study, the genomic RNA of HEV was detected in a total of 11 donors with an ALT level of 63–2598 IU/l among the 4019 donors tested. When stratified by the year group of blood collection, the prevalence of HEV viremia among blood donors with ALT of ≥61 IU/l between 2004 and 2006 was comparable to that in 1998 (0.2 vs. 0.3%), and the prevalence of HEV viremia among blood donors with ALT of ≥201 IU/l was not statistically different among the three year groups of 1991–1995, 1996–1999, and 2004–2006 (1.3, 3.4, and 3.3%, respectively), or from that of 2002–2003 (4.4% or 1/23) [7], suggesting that de novo subclinical HEV infection occurred at an almost constant rate during the last 16 years in Tochigi Prefecture, Japan. In industrialized countries including Japan, maintenance of good hygiene of the water supply and sewage systems made the likelihood of waterborne infection of hepatitis A virus (HAV) extremely low [13]. However, our observations are consistent with the notion that transmission of HEV would not

be prevented by only improvement of sanitary conditions, despite the lower infectivity and transmissibility of HEV than HAV [36].

Domestically acquired hepatitis E has been reported in industrialized countries including the United States and European countries since 1997 [2, 5, 10, 15, 21, 34, 35, 37, 55]. However, only a limited number of sporadic cases of acute hepatitis E have been reported in the United States and European countries, and the changing profiles of clinical and subclinical HEV infection have not been studied in these countries. In Japan, clinical hepatitis E is rare compared with clinical hepatitis A but is occurring more frequently than previously thought [30, 33], where the first case of autochthonous hepatitis E was reported in 2001 [43], and presumably indigenous HEV strains have been recovered from individuals who contracted HEV infection in the 1970s [27, 28]. It remains unknown, however, why the prevalence of domestic HEV infection has remained stable during the last few decades in Japan. It has recently been suggested that zoonotic foodborne transmission of HEV from domestic pigs and wild boars to humans plays an important role in the occurrence of cryptic hepatitis E in Japan, where Japanese people have distinctive habits of eating raw fish (sushi or sashimi) and, less frequently, uncooked or undercooked meat (including the liver and colon/intestine of animals) [17, 23, 31, 41, 57]. Of note, we found a high prevalence of swine anti-HEV antibodies and a high HEV viremia rate among Japanese pigs [46, 48]. The majority of patients with sporadic acute hepatitis E in Hokkaido had a history of consuming grilled or undercooked pig liver and/or intestine approximately 2–8 weeks prior to the onset of hepatitis E [31]. Pig liver specimens from 7 (1.9%) of 363 packages sold in local grocery stores in Hokkaido had detectable HEV RNA [57]. These results strongly suggest that consumption of undercooked pig liver/intestine is a potential risk factor for HEV infection. Transfusion-associated hepatitis E has also been reported in Japan [22, 27]. Recently, of interest, it was reported that HEV RNA was detected in bivalves called Yamato-Shijimi (*Corbicula japonica*) obtained from Japanese rivers, indicating that HEV contaminates river water in Japan [18]. However, the mode of HEV transmission in the 11 viremic donors in the present study was unclear. Further studies are needed to elucidate the mode(s) of clinical and subclinical HEV infection in the general population of Japan including Tochigi Prefecture.

As the 11 viremic donors identified in the present study had an elevated ALT level, the blood from the 11 donors was not used for transfusion, suggesting that ALT testing helps prevent transfusion-transmitted HEV infection. As one of the 11 infected donors had only a slightly elevated ALT level of 63 IU/l, it seems likely that even donors with a normal ALT level ($\leq$60 IU/l) may have detectable HEV RNA. The prevalence of HEV RNA decreased with the ALT level and was significantly less frequent among the 3000 donors with ALT of 61–100 IU/l than among the 93 donors with ALT of $\geq$201 IU/l (0.067 vs. 4.3%, $P<0.0001$) in 1998 and 2004–2006. Although the number of donors tested was limited, it is reasonable to speculate that the prevalence of ongoing HEV infection among donors with a normal ALT level may be less than 0.067% in Tochigi Prefecture. The proportion of such donors may be very small or negligible, particularly in the southern part of Japan, where only 1.7% (9/527) of blood donors with ALT of $\geq$61 IU/l had anti-HEV IgG [7].

Reflecting the polyphyletic nature of human and animal HEV isolates of Japanese origin [30, 33, 44], the HEV isolates recovered from the 11 viremic donors in the present study differed by 1.7–22.8% from each other and segregated into genotype 3 or 4. Ten human HEV strains of genotype 3 (HE-JI3 [AB080579], HE-JBD1 [AB112743], HE-JBD2 [AB154829], HE-JBD3 [AB154830], and 6 unpublished isolates) have been isolated in the same prefecture as that of the 11 viremic donors and shared identities ranging from 80.6 to 99.8% with the 8 genotype 3 HEV isolates obtained in the present study. As for human HEV strains of genotype 4, two strains (HE-JI4 [AB080575] and HE-JK4 [AB099347]) isolated in the same prefecture shared 87.9–99.3% identities with the 3 genotype 4 HEV isolates obtained in the present study. These results further support the marked heterogeneity of the HEV genome and its wide distribution in Japan, even within a certain prefecture in this country.

In conclusion, 11 blood donors with HEV viremia were identified among 4019 voluntary blood donors with an elevated ALT level at a blood center located in the northern part of mainland Honshu of Japan, where hepatitis E is low-endemic. In this study, 2.4% of individuals with ALT of ≥201 IU/l had ongoing subclinical infection of various HEV strains, and the prevalence of HEV viremia was distributed nearly evenly in the year groups of 1991–1995, 1996–1999, and 2004–2006, suggesting that the occurrence rate of subclinical infection with divergent HEV strains has essentially remained unchanged during 1991–2006 in Japan. Future studies are warranted to clarify the mode(s) of HEV transmission that may be responsible for the stable occurrence of clinical and, mostly, subclinical HEV infections over the past several decades in humans living in industrialized countries, where a significant proportion of the general population have HEV antibodies, but hepatitis E is believed to be non- or low-endemic.

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研究報告の概要

【目的と意義】
E 型肝炎は人獣共通感染症として認識されており、E 型肝炎ウイルス (HEV) に感染した動物の生肉を食することによるヒトへの感染が、我が国における第 1 の感染経路となっている。また、感染ドナーからの輸血による 2 次感染例も報告されている。そのため、これらの感染経路における安全施策が緊要な課題となっている。ウイルスに対する安全対策として、血液製剤の製造工程には、ウイルス除去膜によるろ過や液状加熱処理が導入されている。私達は、ウイルス除去膜や液状加熱に対する HEV の性状を調査することにより、HEV への安全対策における基礎データの取得を試みた。
【材料と方法】
HEV に感染した豚糞便より精製した、ORF2 領域の Genome sequencing において cluster の異なる 4 種類の HEV を得た。これらをスパイクウイルスとして使用し、PLANOVA75N、35N、20N 及び 15N (旭化成メディカル) を用いたろ過実験による HEV の挙動について検討した。ウイルス量はろ過前後の HEV RNA ゲノム量を RT-PCR にて測定し、各 HEV の PLANOVA による除去効果を調べた。また、PBS 及びアルブミン含有溶液組成中で 60℃の加熱を行い、0、0.5、1、2 及び 5 時間目の感染能の推移についても調べた。ウイルスの感染能については、希釈系列を作成したウイルス液を A549 細胞 (ヒト肺癌細胞) に感染させ、7 日間培養した後、細胞中の HEV-RNA を RT-PCR により検出した。
【結果と考察】
ウイルス除去膜によるろ過実験においては、4 株とも PLANOVA15N 及び 20N で検出限界以下にまで HEV が除去されることが確認され、これまで報告されている HEV の粒子径とほぼ一致する挙動を示した。また、HEV の液状加熱では、溶液組成により HEV の不活化効果に差が生じることが示唆された。すなわち、4 株とも PBS 組成では加熱開始後短時間で検出限界以下まで不活化されたが、アルブミン存在下においては、4 株とも加熱開始後 5 時間目においても測定限界以下にまで不活化されることはなかった。これまで HEV は熱に弱いと考えられていたが、溶媒組成や共存タンパク等によって保護作用が生じ、条件によって異なる不活化効果を示すことが示唆された。この結果は、血液製剤や加工食品において慎重に本ウイルスの不活化効果を検討しなければならないことを示している。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリン－IH の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の破傷風抗毒素を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理 (ナノフィルトレーション) を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| これまで HEV は熱に弱いと考えられていたが、アルブミン存在下の 60℃、5 時間処理では検出限界以下まで不活化されなかったとの報告である。<br>本剤から HEV が伝播したとの報告はない。万一原料血漿に HEV が混入したとしても、EMC および CPV をモデルウイルスとしたウイルスバリデーション試験成績から、製造工程において十分に不活化・除去されると考えている。 | モデルウイルスを用いたウイルスバリデーション試験に加えて、必要に応じて実ウイルスを用いた工程評価を実施する。 |

(A)

## 2P206

### カンボジアにおける河川と地下水からの腸管系ウイルスの検出

松原康一[1]、北島正章[1]、原本英司[2]、片山浩之[1]、大垣眞一郎[1]

東京大学　工学系研究科　都市工学専攻[1]、
国立保健医療科学院　水道工学部[2]

matsubara@env.t.u-tokyo.ac.jp

【目的と意義】

ロタウイルスやノロウイルスなど腸管系ウイルスの開発途上国における重要性が明らかになりつつあるが、疫学情報システム整備の未発達のためウイルスの流行や感染リスクについて得られる情報は限られている。環境水中からのウイルス検出は、生活用水の直接摂取による感染リスクの定量が可能な点、家庭からの排水が集まる河川等において地域のウイルス発生・流行を把握することが可能な点で非常に重要である。本研究では、環境水試料からのウイルス濃縮法を途上国での調査に対して適応し、カンボジアにおける水環境のウイルス汚染状況調査を行った。

【材料と方法】

カンボジア・シェムリアップ州を中心に地下水および河川水を採水し、河川水 50mL、地下水 1L をマグネシウム添加・酸洗浄・アルカリ誘出法 (Katayama et al., 2002 Appl. Environ. Microbiol. 68:1033-1039) によって 5mL まで現地での濃縮を行った。対象地域の地下水および河川水に含まれる粘土質の懸濁成分による膜の目詰まりを解消するため、濃縮時にガラスファイバーろ紙 (GF/D, Whatman) を用いて前ろ過する改良を加えた。濃縮した試料は東京大学の実験室まで持ち帰り、RNA 抽出および逆転写、または DNA 抽出の後、TaqMan PCR に供しウイルスを検出した。また、糞便性汚染指標として大腸菌・大腸菌群を mColiBlue broth (Millipore) を用いて現地にて分析した。

【結果と考察】

地下水および河川水から、E 型肝炎ウイルス (陽性率 1/10)、A 群ロタウイルス (同 2/10)、腸管アデノウイルス (同 1/10)、A 型肝炎ウイルス (同 1/10)、ノロウイルス G1 型、G2 型 (同各 1/10)、エンテロウイルス (同 1/10) が検出された。大腸菌・大腸菌群濃度が高い地点とウイルスの検出状況は一致しなかった。本研究はカンボジアにおいて環境水中から TaqManPCR によって E 型肝炎ウイルスを検出した最初の報告であり、地域に感染者が存在し潜在的なリスクがあることを示すものである。環境水中に非常に低濃度で存在したと考えられるウイルスを検出できたことから、本研究で用いたウイルス濃縮・検出方法は遠隔地での水系ウイルスのモニタリングに対しても簡便かつ高感度に水中ウイルスを検出する方法として有効であることが示された。

BENE2007-022

## 2P207

### E型肝炎ウイルスの液状加熱及びウイルス除去膜による除去実験

田中宏幸[1]、服部眞次[1]、辻川宗男[1]、久保　純[1]、浦山　健[1,2]、柚木幹弘[1,2]、安江　博[3]、荻原克郎[1]、生田和良[2]

株式会社ベネシス　研究開発本部・枚方研究所[1]、
大阪大学　微生物病研究所　ウイルス免疫分野[2]、
独立行政法人農業生物資源研究所[3]、
酪農学園大学　獣医学部[4]

Tanaka.Hiroyuki@mh.m-pharma.co.jp

【目的と意義】

E 型肝炎は人獣共通感染症として認識されており、E 型肝炎ウイルス (HEV) に感染した動物の生肉を食することによるヒトへの感染が、我が国における第 1 の感染経路となっている。また、感染ドナーからの輸血による 2 次感染例も報告されている。そのため、これらの感染経路における安全施策が緊要な課題となっている。ウイルスに対する安全対策として、血液製剤の製造工程には、ウイルス除去膜によるろ過や液状加熱処理が導入されている。私達は、ウイルス除去膜や液状加熱に対する HEV の性状を調査することにより、HEV への安全対策における基礎データの取得を試みた。

【材料と方法】

HEV に感染した豚糞便より精製した、ORF2 領域の Genome sequencing において cluster の異なる 4 種類の HEV を得た。これらをスパイクウイルスとして使用し、PLANOVA75N、35N、20N 及び 15N (旭化成メディカル) を用いたろ過実験による HEV の挙動について検討した。ウイルス量はろ過前後の HEV-RNA ゲノム量を RT-PCR にて測定し、各 HEV の PLANOVA による除去効果を調べた。また、PBS 及びアルブミン含有溶液組成中で 60℃の加熱を行い、0、0.5、1、2 及び 5 時間目の感染能の推移についても調べた。ウイルスの感染能については、希釈系列を作成したウイルス液を A549 細胞 (ヒト肺癌細胞) に感染させ、7 日間培養した後、細胞中の HEV-RNA を RT-PCR により検出した。

【結果と考察】

ウイルス除去膜によるろ過実験においては、4 株とも PLANOVA15N 及び 20N で検出限界以下にまで HEV が除去されることが確認され、これまで報告されている HEV の粒子径とほぼ一致する挙動を示した。また、HEV の液状加熱では、溶液組成により HEV の不活化効果に差が生じることが示唆された。すなわち、4 株とも PBS 組成では加熱開始後短時間で検出限界以下まで不活化されたが、アルブミン存在下においては、4 株とも加熱開始後 5 時間目においても測定限界以下にまで不活化されることはなかった。これまで HEV は熱に弱いと考えられていたが、溶媒組成や共存タンパク等によって保護作用が生じ、条件によって異なる不活化効果を示すことが示唆された。この結果は、血液製剤や加工食品において慎重に本ウイルスの不活化効果を検討しなければならないことを示している。

別紙様式第2-1

No. 14

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 10. 2 | 該当なし | |

| 一般的名称 | 人赤血球濃厚液 | 研究報告の公表状況 | ProMED 20070930-3228, 2007 Sep 30. 情報源:The Sunday Mail (Qld), 2007 Sep 29. | 公表国 オーストラリア |
|---|---|---|---|---|
| 販売名(企業名) | 赤血球濃厚液-LR「日赤」(日本赤十字社)<br>照射赤血球濃厚液-LR「日赤」(日本赤十字社) | | | |

**研究報告の概要**

○ロスリバーウイルス感染症例急増
オーストラリア、クイーンズランド州で異常発生した蚊がロスリバーウイルス感染を拡大させている。過去4週間の症例数は93例で、過去5年間の同期間の平均32例の約3倍まで増加している。このウイルスは通常は北部の暑い地方で流行しているが、南のブリスベーン地区の過去4週間の感染者数は31例と、前年同時期の7例と比較して4.5倍に達した。これは、通常症例数が最高となる晩夏から初秋の時期と同等であり、この時期としては異常に多い。保健当局は、長い乾期と8月末の季節はずれの土砂降りによって、蚊の産卵時期が3ヵ月早まったと伝えている。
ロスリバーウイルス感染の症状は微熱、紅斑、関節痛である。ワクチンや治療法はなく、罹患した場合は症状が治まるのを待つしかないが、回復までに3ヶ月以上かかる場合もある。人から人へ感染することはないが、蚊の媒介で動物から人に感染が拡大する。
当局は人々に蚊に刺されないように注意することと、溜まり水の除去などの対策を行うよう呼びかけた。蚊の成長を阻害するホルモン剤の散布が実施されている。

| 報告企業の意見 | 今後の対応 |
|---|---|
| オーストラリア、クイーンズランド州で異常発生した蚊が、ロスリバーウイルス感染を拡大させているとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Back

**Archive Number** 20070930.3228
**Published Date** 30-SEP-2007
**Subject** PRO/AH/EDR> Ross River virus - Australia (QLD)

*ROSS RIVER VIRUS - AUSTRALIA (QUEENSLAND)*
**********
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: 30 Sep 2007
Source: The Sunday Mail (Qld) [edited]
<http://www.news.com.au/couriermail/story/0,23739,22502731-3102,00.html>

Unusually high numbers of mosquitoes are spreading the debilitating Ross River virus across the state, with the number of cases soaring by almost 300 percent.

The virus is usually more prevalent in the tropical north, but figures show the number of people infected in the southern Brisbane area in the past 4 weeks is almost 450 percent higher than in the same period last year [2006].

Health bosses are warning people to take precautions against being bitten and are stepping up spraying programs to tackle the problem. They say the long dry spell and the unseasonable downpours at the end of last month [August 2007] have caused mosquitoes to breed 3 months early.

New figures released to The Sunday Mail by Queensland Health reveal there were 93 reported cases of Ross River infection in the past 4 weeks, compared with an average 32 cases in the same period for each of the past 5 years [2002-2006].

North of Mackay, 30 cases were reported; 32 in the central area, which takes in central Queensland and extends south to Brisbane, north of the river; and 31 cases were reported in the southern area, which runs from south of the river in Brisbane to the New South Wales border.

The southern Brisbane figures compare with just 7 in the same period last year [2006]. Dozens more cases are believed to have gone unrecorded or undiagnosed.

Queensland Health said the numbers were unusually high for this time of year, with the highest numbers usually occurring in late summer and early autumn.

Symptoms of Ross River virus include a mild fever, rash, and joint pain, which is similar to arthritis.

Dr Michael Whitby, an infection spokesman for the Australian Medical Association Queensland, said symptoms could vary but the disease could often become debilitating, causing people to take months off work.

"Unfortunately there is no vaccine available, and the arthritis treatment doesn't often do any good," he said. "All people can really do is to wait until it goes away. This can take 3 months or sometimes even longer."

The infection cannot be spread from human to human but can be spread

from animals to humans via mosquitoes. It is confirmed with a blood test taken by a GP.

Councils across the state are already spending millions in a bid to control the mosquitoes before the summer hits. Aerial and land-based spraying has already been carried out across the state, on tidal drains and sites near water.

Health officers at Brisbane City Council have launched an AUD 3.4 million [USD 3 million] mosquito prevention program after receiving about 120 complaints about mosquitoes since 1 Sep 2007, with most from residents in suburbs beside salt marsh areas from Deagon to Wynnum West.

A Brisbane City Council spokesman said: "The rain in August 2007 generated widespread hatching of Brisbane's salt marsh mosquitoes across all the tidal areas from Brighton to Tingalpa. There has also been much activity from a range of freshwater breeding mosquito species right across the city and southeast Queensland."

The spokesman urged residents to be vigilant around their homes. "Container-breeding mosquitoes are very active right now after the rain and will use any receptacle that holds water," he said. "We advise that any drums or buckets being used to store water should be covered, and any rubbish that holds water should be discarded. Mosquito screens on tanks, on both inlet and overflow pipes, should also be checked and kept in place."

*On the Sunshine Coast, councils are dumping large amounts of* hormone-laced sand on mosquito breeding grounds as part of an AUD one million [USD 885 000] outbreak prevention project.

The hormone, which stunts the growth of juvenile mosquitoes but doesn't harm other insects, was dropped in Caloundra, Buderim, Bli-Bli, Coolum, Noosa, Noosa's North Shore and Peregian Beach.

[Byline: Hannah Davies and Lou Robson]

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Communicated by:
ProMED-mail <promed@promedmail.org>

[Mod. CP provided an excellent summary of the Ross River virus situation in Australia in archive no. 20040403.0916:

"Epidemics of benign polyarthritis were recorded in Australia as early as 1927, and the etiologic agent was isolated in 1963. Ross River virus was shown to be a mosquito-transmitted virus belonging to the genus _Alphavirus_ of the family _Togaviridae_. Ross River virus is endemic in most coastal regions of Australia and since the 1980's appears to have extended its geographical range to include most of the island communities of the South Pacific. The animal reservoir species are various, and humans exhibit a significant viraemia such that some epidemics are maintained in a human-mosquito-human transmission cycle. The mosquito vectors vary according to the local environment. Fortunately, illness in humans -- although occasionally prolonged and painful -- is not fatal, and recovery is complete."

Although recovery from Ross River virus infection is complete, *symptoms may persist for years. With high incidence of Ross River* virus infection early this spring [2007], one wonders whether virus transmission will accelerate as warmer spring to summer conditions progress. ProMED requests further information about this year's outbreak and the effectiveness of hormone (presumably target-specific insect juvenile hormone) wetland treatment for vector mosquito control as it becomes available.

A map of Australia showing the location of Queensland an be accessed at:
<http://www.lib.utexas.edu/maps/australia/australia_pol99.jpg>.
- Mod.TY]

[see also:
2006
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Ross River virus - Australia (02): VIC 20060204.0363
Ross River virus - Australia: NSW, SA 20060114.0138

2005
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Ross River virus - New Zealand (Waikato) ex Australia (NT) 20050120.0195
2004
---
Ross River virus - Australia (QLD) 20040403.0916
Ross River virus - Australia (QLD) (02): background 20040404.0928
Ross River virus - Australia (WA) (03) 20040105.0049
Ross River virus - Australia (WA) (04) 20040427.1165
2003
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Ross River virus - Australia (WA) (02) 20031230.3170
Ross river virus - Australia (WA): alert 20031008.2529
Ross River/Barmah Forest viruses - Australia (NSW) 20030628.1597
2002
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Ross river virus - Australia (Tasmania) (03) 20020821.5105
Ross River Virus - Australia (Tasmania) 20020410.3927
2001
---
Ross River virus - Australia (South) 20010320.0560
Ross River virus - Australia (North. Territory) (03) 20010225.0367
Ross River virus - Australia 20010116.0127
Ross River virus - Australia (Northern Territory) 20010108.0062
2000
---
Ross River virus - Australia (South Australia): ALERT 20001229.2292
Ross River/Barmah virus - Australia (SW): alert 20000123.0115
1999
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Ross River virus - Australia (Tasmania) (02) 19991222.2198
Ross River virus - Australia (West): alert 19991106.1989
Ross River virus - Australia (Tasmania) 19990311.0371
1998
---
Ross River virus infection - Australia 19981231.2472
Ross River virus - Australia (New South Wales) (02) 19980112.0087
Ross River virus - Australia (New South Wales) 19980109.0067
1997
---
Ross River virus, military exercises: Australia 19970728.1587
Ross River fever - Australia (03) 19970605.1172
Ross River, Barmah Forest viruses - Australia 19970604.1162
Ross River fever - Australia (02) 19970604.1161
Ross River fever - Australia (Sydney) 19970602.1126]
.....ty/msp/dk

13th ICID | site map | ISID home

医薬品　研究報告　調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2007年12月25日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | 別紙のとおり | | 研究報告の公表状況 | CDC／Travelers' Health, Outbreak Notice. January 8, 2008. | 公表国<br>ウガンダ | |
| 販売名（企業名） | 別紙のとおり | | | | | |

**研究報告の概要**

問題点：2007年後半にウガンダで発生したエボラ出血熱のアウトブレイクは、既知の4つのエボラウイルス株と異なる新たなウイルス株が原因である可能性がある。

米国CDCとウガンダ保健省は、ウガンダ西部に位置するBundibugyo地区におけるエボラ出血熱のアウトブレイクについて報告した。アウトブレイクは早ければ2007年8月から始まった可能性がある。2008年1月3日までに148人が罹患し、37人が死亡した。症例サンプルの遺伝子解析により、既知の4つのエボラウイルス株と異なる、新たなウイルス株であることが示された。しかし、確定するには更なる解析が必要である。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 別紙のとおり | 今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。 |

使用上の注意記載状況・その他参考事項等

記載なし



MedDRA10.1

(6)

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|---|---|
| 一般的名称 | ①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ |
| 販売名(企業名) | ①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用 |
| 報告企業の意見 | エボラ出血熱はエボラウイルスによる急性熱性疾患であり、ラッサ熱、マールブルグ病、クリミア・コンゴ出血熱とともに、ウイルス性出血熱の一疾患である。エボラウイルスは、フィロウイルス科（*Filoviridae*）に属し、1本鎖RNAを核酸として持ち、エンベロープを有する。短径が80～100nm、長径が700～1,500nmで、多形性（U字状、ひも状、ぜんまい状等）を示す。<br>エボラ出血熱は、現在までアフリカの中央部でのみ発生している。感染者・患者の血液や体液との接触によりヒトからヒトへ感染が拡大し、多数の死者を出す流行を起こす。ヒトは終末宿主であるが、動物、昆虫などの自然宿主、媒介動物については全く不明である。そのため、自然界からヒトへの感染経路も不明である。<br>発症は突発的で進行も早い。潜伏期は2～21日で、汚染注射器を通した感染では早く、接触感染では長い。発熱、頭痛、腹痛、咽頭痛、筋肉痛、胸部痛及び出血等の症状がみられ、重篤化する。致死率は患者の53～88%と高い。<br>弊所の血漿分画製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。<br>各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に従い、ウシウイルス性下痢ウイルス（BVDV）、仮性狂犬病ウイルス（PRV）、ブタパルボウイルス（PPV）、A型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告したエボラウイルスは、エンベロープの有無、核酸の種類等からモデルウイルスとしてはBVDVが該当すると考えられるが、上記バリデーションの結果から、BVDVの除去・不活化効果を有することを確認している。<br>また、これまでに当該製剤によるエボラウイルス感染の報告例は無い。<br>以上の点から、当該製剤はエボラウイルスに対する安全性を確保していると考える。 |

*現在製造を行っていない

Outbreak Notice
Updated: Ebola Outbreak in the District of Bundibugyo, Uganda
This information is current as of today, January 24, 2008 at 20:11

**Updated**: January 08, 2008

The U.S. CDC and the Ministry of Health of Uganda have reported an Ebola hemorrhagic fever outbreak in the Bundibugyo district located in the Western part of the country. The outbreak may have begun as early as August 2007. As of January 3, 2008, 148 people have become ill and 37 people have died. Genetic analysis of samples from case-patients indicated that this is a new virus strain distinct from the four known strains of Ebola virus. However, further studies will be needed before this can be verified.

Ebola hemorrhagic fever is a rare, serious viral disease which develops suddenly, with common symptoms of fever, headache, joint and muscle aches, sore throat, and weakness. Diarrhea, vomiting, and stomach pain start after the first symptoms. A skin rash may develop. By the third or fourth day of illness some people with Ebola hemorrhagic fever may develop internal and external bleeding, shock and organ failure.

Ebola is spread through direct contact with blood or other body fluids (e.g., saliva, urine) of infected persons or objects that have been contaminated with infected body fluids. People who have close contact with a nonhuman primate infected with the virus are also at risk.

## Recommendations for U.S. Travelers

The World Health Organization (WHO) has reported that there is no need for any travel restrictions to Uganda. Generally, the risk of contracting Ebola virus is low for travelers. CDC recommends that anyone traveling to Uganda take the following steps to prevent Ebola virus infection:

- Avoid contact with Ebola patients and their body fluids.
- Avoid touching used needles or other medical waste.
- Avoid contact with wild animals and bushmeat, including primates.

## More Information

For information about the current situation, see the WHO report at www.who.int.

For additional information on Ebola hemorrhagic fever, please see http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm.

To learn more about traveling to areas with hemorrhagic fevers, see the Viral Hemorrhagic Fevers section of *CDC Health Information for International Travel 2008*.

Page Located on the Web at http://wwwn.cdc.gov/travel/contentEbolaUganda.aspx

別紙様式第2-1

No. 20

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 10. 22 | 該当なし | |

| 一般的名称 | 人赤血球濃厚液 | 研究報告の公表状況 | 山田正仁, 篠原もえ子, 野崎一朗, 浜口毅, 中村好一, 北本哲之, 佐藤猛, 水澤英洋, CJDサーベイランス委員会. 2007年プリオン研究会 | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 赤血球濃厚液-LR「日赤」(日本赤十字社)<br>照射赤血球濃厚液-LR「日赤」(日本赤十字社) | | | 日本 |

**研究報告の概要**

○わが国におけるヒトのプリオン病の実態:最近のサーベイランスデータ
我が国の人口動態統計では、クロイツフェルト・ヤコブ病(CJD)による死亡は過去20年以上に渡り右肩上がりに増加傾向を示し、2005年は人口100万対1.23人であった。『プリオン病および遅発性ウイルス感染症に関する調査研究班』・CJDサーベイランス委員会による現行のプリオン病調査は1999年から始まった。そこでは、プリオン病が疑われる全患者について、画像、脳脊髄液マーカー、プリオン蛋白(PrP)遺伝子型、病理などの検査を含めた実地調査を行うことを原則としている。このシステムにより過去8年間に918例がプリオン病と判定された。病型別では、孤発性CJD716例(78.0%)、遺伝性プリオン病128例(14.0%)、感染性(獲得性)CJD72例(7.8%)[変異型CJD(vCJD)1例/硬膜移植後CJD(dCJD)71例]、および分類不能2例(0.2%)であった。PrP遺伝子に変異がないことを確認した孤発性CJD387例の臨床像をみると、進行が速く(無動性無言まで9ヵ月未満)特徴的な脳波所見を有する典型例は74%、それ以外の非典型例が26%を占めた。進行が遅く特徴的脳波を欠く、最も非典型的な群は、他群と較べて脳脊髄液マーカーやMRI上の高信号の陽性率も低く、Parchi分類でMM2型に属し、特に視床型が臨床診断上問題であった。遺伝性プリオン病128例の分類では、コドン180変異42例(32.9%)、コドン200変異CJD26例(20.4%)、コドン102変異25例(19.6%)、コドン232変異17例(13.3%)他の順であり、欧米ではほとんどないコドン180、232変異が多くみられるなどの特色があった。dCJDは1996年の佐藤班による全国調査以来、硬膜移植歴が判明したものを合計すると129例になった。dCJDの中で、比較的緩徐な進行を示し特徴的脳波を欠き脳にPrP斑を認める非典型例(プラーク型)の割合は剖検例では48%であり、臨床例を含めるとdCJD全体の約1/3を占めると考えられた。2007年7月現在、vCJDは英国短期滞在歴がある1例のみである。

| 報告企業の意見 | 今後の対応 |
|---|---|
| CJDサーベイランス委員会による調査では過去8年間に日本国内で918例がプリオン病と判定された。また、人口動態統計ではCJDによる死亡者数は過去20年以上に亘って増加傾向を示し、2005年は人口100万対1.23人であったとの報告である。 | 日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980～96年に1日以上の英国滞在歴のある方からの献血を制限している。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(1)

# わが国におけるヒトのプリオン病の実態：最近のサーベイランスデータ

山田正仁[1,6]、篠原もえ子[1]、野崎一朗[1]、浜口　毅[1]、中村好一[2,6]、北本哲之[3,6]、佐藤　猛[4,6]、水澤英洋[5,6]、CJD サーベイランス委員会[6]

[1]金沢大学大学院　脳老化・神経病態学（神経内科）、[2]自治医大公衆衛生、[3]東北大学大学院プリオン蛋白研究部門、[4]東大和病院、[5]東京医科歯科大学大学院脳神経病態学（神経内科）、[6]厚生労働省・難治性疾患克服研究事業「プリオン病及び遅発性ウイルス感染症に関する調査研究班」・CJD サーベイランス委員会

わが国の人口動態統計では、クロイツフェルト・ヤコブ病（CJD）による死亡は過去 20 年以上に渡り右肩上がりに増加傾向を示し、2005 年は人口 100 万対 1.23 人であった。『プリオン病および遅発性ウイルス感染症に関する調査研究班』・CJD サーベイランス委員会による現行のプリオン病調査は 1999 年から始まった。そこでは、プリオン病が疑われる全患者について、画像、脳脊髄液マーカー、プリオン蛋白（PrP）遺伝子型、病理などの検査を含めた実地調査を行うことを原則としている。このシステムにより過去 8 年間に 918 例がプリオン病と判定された。病型別では、孤発性 CJD 716 例（78.0%）、遺伝性プリオン病 128 例（14.0%）、感染性（獲得性）CJD 72 例（7.8%）[変異型 CJD（vCJD）1 例／硬膜移植後 CJD（dCJD）　71 例]、および分類不能 2 例（0.2%）であった。PrP 遺伝子に変異がないことを確認した孤発性 CJD 387 例の臨床像をみると、進行が速く（無動性無言まで 9 ヶ月未満）特徴的な脳波所見を有する典型例は 74%、それ以外の非典型例が 26%を占めた。進行が遅く特徴的脳波を欠く、最も非典型的な群は、他群と較べて脳脊髄液マーカーや MRI 上の高信号の陽性率も低く、Parchi 分類で MM2 型に属し、特に視床型が臨床診断上問題であった（Hamaguchi *et al. Neurology* 64:643, 2005）。遺伝性プリオン病 128 例の分類では、コドン 180 変異 42 例（32.9%）、コドン 200 変異 CJD 26 例（20.4%）、コドン 102 変異 25 例（19.6%）、コドン 232 変異 17 例（13.3%）他の順であり、欧米ではほとんどないコドン 180、232 変異が多くみられるなどの特色があった。dCJD は 1996 年の佐藤班による全国調査以来、硬膜移植歴が判明したものを合計すると 129 例になった。dCJD の中で、比較的緩徐な進行を示し特徴的脳波を欠き脳に PrP 斑を認める非典型例（プラーク型）の割合は剖検例では 48%であり、臨床例を含めると dCJD 全体の約 1/3 を占めると考えられた（Noguchi-Shinohara *et al. Neurology* 69:360, 2007）。2007 年 7 月現在、vCJD は英国短期滞在歴がある 1 例（Yamada *et al. Lancet* 367:874, 2006）のみである。

医薬品　研究報告　調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2007年12月17日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | 別紙のとおり | | 研究報告の公表状況 | PloS Pathogens. 2007;3:1895-1906 | 公表国<br>インド洋南西地域およびインド | |
| 販売名（企業名） | 別紙のとおり | | | | | |

**研究報告の概要**

問題点：2005年から2006年にかけてのレユニオン諸島でのチクングニヤウイルス感染のアウトブレイクは、ヒトスジシマカをチクングニヤウイルスのベクターとし、また致命的な感染が報告された流行であるという特徴を持っていた。

以前の流行では、チクングニヤウイルス（CHIKV）感染は非致死性の感染症と考えられていた。しかし、レユニオン諸島でアウトブレイクしたCHIKV感染は、266,000人が発症し、260人の死者が出た。CHIKVは、*Aedes aegypti*（ネッタイシマカ）をプライマリーベクターとするが、2005～2006年のレユニオン諸島でのアウトブレイクにおけるベクターは*Aedes albopictus*（ヒトスジシマカ）であった。
研究者らは、CHIKVのエンベロープ蛋白(E1)の226番目のアミノ酸がアラニンからバリンに変異していることを明らかにした。この変異により、CHIKVはネッタイシマカと比較して、ヒトスジシマカへの感染性が増し、その唾液腺でより早く増殖するようになり、また乳のみマウスへもより効率的に感染するようになった。
一つのアミノ酸置換がベクターの特異性に影響を与えるという今回の結果は、通常のベクターが存在しない地域で変異ウイルスが流行を起こした理由をうまく説明をしている。これは、ウイルスが新しい地域に入り込んだときにどのように感染サイクルを確立するかに関する重要な仮説となる。ヒトスジシマカは広く分布しているため、この変異はCHIKVの分布が欧州やアメリカ大陸に拡がる可能性を増大させることとなる。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 別紙のとおり | 今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。 |

使用上の注意記載状況・その他参考事項等

記載なし

| | |
|---|---|
| 一般的名称 | ①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ |
| 販売名(企業名) | ①献血アルブミン 20 “化血研”、②献血アルブミン 25 “化血研”、③人血清アルブミン “化血研” *、④ “化血研” ガンマーグロブリン、⑤献血静注グロブリン “化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500 注射用 |
| 報告企業の意見 | チクングニヤウイルス (Chikungunya virus) は、トガウイルス科 (*Togaviridae*) のアルファウイルス属 (*Alphavirus*) に分類される 1 本鎖の RNA を核酸として持つ直径 70nm のエンベロープを有する球状粒子である。いままでに日本国内での感染・流行はないが、2006 年 12 月に海外からの輸入症例 2 例が報告された。チクングニヤウイルスは蚊によって媒介されるが、感染後ウイルス血症を起こすことから、血液を介してウイルス感染する可能性を完全に否定できないため本報告を行った。<br>弊所の血漿分画製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去・不活化工程が存在しているので、仮にウイルスが原料血漿に混入していたとしても、ウイルスクリアランスが期待される。各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン (医薬発第 1047 号、平成 11 年 8 月 30 日)」に従い、ウシウイルス性下痢ウイルス (BVDV)、仮性狂犬病ウイルス (PRV)、ブタパルボウイルス (PPV)、A 型肝炎ウイルス (HAV) または脳心筋炎ウイルス (EMCV) をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告したチクングニヤウイルスはエンベロープの有無、核酸の種類等からモデルウイルスとしては BVDV が該当すると考えられるが、上記バリデーションの結果から、BVDV の除去・不活化効果を有することを確認している。<br>また、これまでに弊所の血漿分画製剤によるチクングニヤウイルス感染の報告例は無い。<br>以上の点から、当該製剤はチクングニヤウイルスに対する安全性を確保していると考える。 |

*現在製造を行っていない

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# A Single Mutation in Chikungunya Virus Affects Vector Specificity and Epidemic Potential

**Konstantin A. Tsetsarkin, Dana L. Vanlandingham, Charles E. McGee, Stephen Higgs***

Department of Pathology, University of Texas Medical Branch, Galveston, Texas, United States of America

**Chikungunya virus (CHIKV) is an emerging arbovirus associated with several recent large-scale epidemics. The 2005–2006 epidemic on Reunion island that resulted in approximately 266,000 human cases was associated with a strain of CHIKV with a mutation in the envelope protein gene (E1-A226V). To test the hypothesis that this mutation in the epidemic CHIKV (strain LR2006 OPY1) might influence fitness for different vector species, viral infectivity, dissemination, and transmission of CHIKV were compared in *Aedes albopictus*, the species implicated in the epidemic, and the recognized vector *Ae. aegypti*. Using viral infectious clones of the Reunion strain and a West African strain of CHIKV, into which either the E1–226 A or V mutation was engineered, we demonstrated that the E1-A226V mutation was directly responsible for a significant increase in CHIKV infectivity for *Ae. albopictus*, and led to more efficient viral dissemination into mosquito secondary organs and transmission to suckling mice. This mutation caused a marginal decrease in CHIKV *Ae. aegypti* midgut infectivity, had no effect on viral dissemination, and was associated with a slight increase in transmission by *Ae. aegypti* to suckling mice in competition experiments. The effect of the E1-A226V mutation on cholesterol dependence of CHIKV was also analyzed, revealing an association between cholesterol dependence and increased fitness of CHIKV in *Ae. albopictus*. Our observation that a single amino acid substitution can influence vector specificity provides a plausible explanation of how this mutant virus caused an epidemic in a region lacking the typical vector. This has important implications with respect to how viruses may establish a transmission cycle when introduced into a new area. Due to the widespread distribution of *Ae. albopictus*, this mutation increases the potential for CHIKV to permanently extend its range into Europe and the Americas.**

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## Introduction

The large-scale epidemic of the mosquito-transmitted alphavirus, Chikungunya virus (CHIKV), began in Kenya in 2004 and spread to several Indian Ocean islands including the Comoros, Mauritius, the Seychelles, Madagascar, Mayotte and Reunion. On Reunion island alone there were approximately 266,000 cases (34% of the total island population) [1–6]. In the continuing Indian epidemic there have been at least 1.4M cases reported [7–10] with continued expansion in Sri Lanka and Indonesia. CHIKV had not been reported to cause fatalities in prior outbreaks; however, during the outbreak on Reunion island, CHIKV was associated with at least 260 deaths [11,12]. The strain of CHIKV responsible for the Indian Ocean island epidemic has been well-characterized in cell culture and mosquito models [13–15]; however, the underlying genetic basis of the atypical phenotype of this CHIKV strain remains unknown.

CHIKV is transmitted by *Aedes* species mosquitoes, primarily *Ae. aegypti*. However, the 2005–2006 CHIKV epidemic on Reunion island was unusual because the vector responsible for transmission between humans was apparently the Asian tiger mosquito, *Ae. albopictus* [3,16]. This conclusion is based on several factors. This species is known to be susceptible to CHIKV infection and although infectious virus was not isolated from *Ae. albopictus* during the epidemic, CHIKV RNA was detected (X. de Lamballerie, personal communication). Furthermore, the species is anthropophylic, was abundant during the epidemic, and other potential vectors specifically *Ae. aegypti* were relatively scarce with a very limited distribution (P. Reiter, personal communication). *Ae. albopictus* is abundant and widely distributed in urban areas of Europe and the United States of America [17–22]. CHIKV infections have been reported in many travelers returning to the US and Europe [12,23–26] causing concern that the virus could be introduced and become established in these areas [1,27,28]. In August and September of 2007, a CHIKV-*Ae. albopictus* transmission cycle was reported for the first time in Europe, with an estimated 254 human cases occurring in Italy [29,30].

Alphaviruses are enveloped single stranded positive sense RNA viruses. Genomic RNA, of ≈ 12,000 nt, encodes four non-structural (ns1–4) and three main structural proteins (capsid, E2 and E1). At neutral pH, E2 and E1 exist as heterodimers in which E2 forms spikes on the virion surface that interact with cellular receptors. The E1 protein lies below E2 and mediates fusion of the viral and cellular membranes during viral entry [31].

Analysis of CHIKV genome microevolution during the 2005–2006 Indian Ocean epidemic identified an alanine to valine mutation at position 226 in the E1 envelope glycoprotein (E1-A226V) among viral isolates obtained during the

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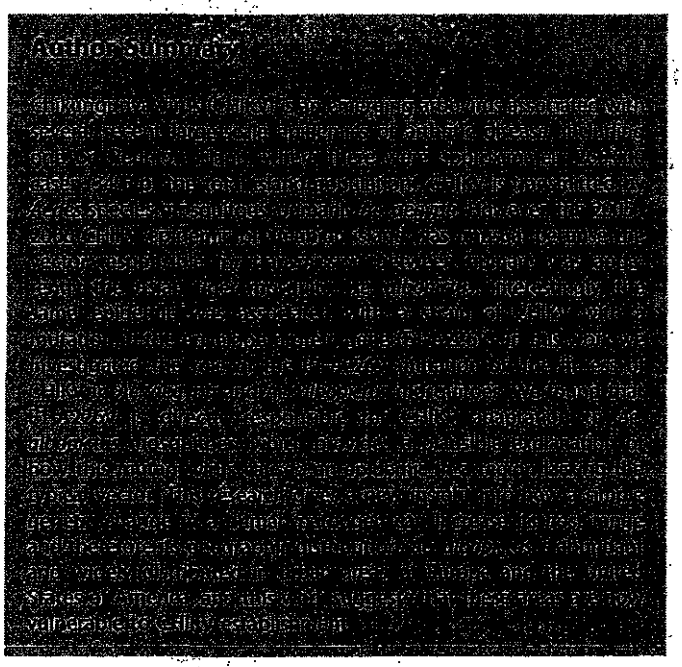
* To whom correspondence should be addressed. E-mail: sthiggs@utmb.edu



outbreak [32]. The reason for this was unclear but it was hypothesized that the E1-A226V mutation might influence infectivity of CHIKV for mosquito vectors [11,32]. Interestingly, earlier studies have identified that a P→S mutation in the same position of the E1 glycoprotein is responsible for the modulation of Semliki Forest virus's (SFV, a member of the alphavirus family) requirements for cholesterol in the target membrane [33]. It also has been shown that the presence of this mutation results in more efficient growth of SFV in *Ae. albopictus* mosquitoes [34]. However, no evidence has been presented to directly correlate the release from the cholesterol dependence, associated with the E1-P226S mutation in SFV, with a growth advantage in *Ae. albopictus*. It is unknown if dependence on cholesterol for growth in mosquito cells is a requirement of all alphaviruses.

To test the hypothesis that the E1-A226V mutation might influence the fitness of CHIKV in mosquito vectors, we compared the effect of this mutation on CHIKV mosquito infectivity, the ability to disseminate into heads and salivary glands, and the relative fitness in competition assays for transmission by *Ae. albopictus* and *Ae. aegypti* to suckling mice. We also analyzed the effect of the E1-A226V mutation on CHIKV cholesterol dependence for growth in mosquito C6/36 (*Ae. albopictus*) cells. Here we report findings that a single nucleotide change, which arose during the epidemic, significantly increases fitness of the virus for *Ae. albopictus* mosquitoes and was associated with CHIKV dependence on cholesterol in the mosquito cell membrane. This change likely enhanced CHIKV transmission by an atypical vector and contributed to the maintenance and scale of the epidemic.

## Results

### Effect of E1 A226V Mutation on Fitness of CHIKV in *Ae. albopictus* Mosquitoes

To test the hypothesis that the E1-A226V mutation altered CHIKV infectivity for *Ae. albopictus* mosquitoes, CHIKV infectious clones derived from an epidemic Reunion island human isolate were used [15], including one clone (LR-GFP-226V) expressing enhanced green fluorescent protein (eGFP). Clones were further engineered to express E1 protein containing an alanine at position E1–226 (LR-GFP-226A) representing the CHIKV genotype prevalent prior to the outbreak gaining momentum (Figure S1). RNAs produced from both clones (LR-GFP-226V and LR-GFP-226A) have comparable specific infectivity values, produced similar viral titers following transfection into BHK-21 cells (Table S1) and have similar growth kinetics in mosquito (C6/36) and mammalian (BHK-21) cells lines (Figure S2A and S2B).

The relative infectivity of LR-GFP-226V and LR-GFP-226A viruses was analyzed in female *Ae. albopictus* mosquitoes orally exposed to serial 10-fold dilutions of CHIKV (LR-GFP-226 V or A). To determine whether infection rates correlate with blood meal titer, midguts dissected from mosquitoes at 7 days post-infection (dpi) were analyzed for foci of eGFP-expressing cells by fluorescence microscopy (Figure 1A; Table 1). In two independent experiments, LR-GFP-226V virus was found to be approximately 100-fold more infectious to *Ae. albopictus* than LR-GFP-226A virus ($p<0.01$). To test if the infectivity phenotype was directly linked to the mutation, the complementary reverse mutation, E1-A226V, was introduced into an infectious clone of a West African CHIKV strain, 37997-GFP (37997-GFP-226A) (Figure S1). The Reunion and 37997 strains of CHIKV are distantly related, with only 85% nucleotide sequence identity. The parental 37997-GFP-226A and the 37997-GFP-226V viruses were indistinguishable in cell culture experiments (Table S1; Figure S2C and S2D); however, *in vivo* experiments in *Ae. albopictus* mosquitoes revealed that the E1-A226V mutation significantly decreases the oral infectious dose 50 ($OID_{50}$) value for the 37997-GFP-226V virus ($p<0.01$) to an extent similar to that observed for LR-GFP-226V virus (Figure 1B; Table 1). These data conclusively demonstrate that the single E1-A226V point mutation is therefore sufficient to significantly reduce the $OID_{50}$ of the 37997-GFP virus ($p<0.01$) in *Ae. albopictus* mosquitoes equivalent to that observed for the LR-GFP-226V virus (Figure 1A; Table 1).

To further evaluate viral fitness of the epidemic CHIKV E1-A226V mutation in *Ae. albopictus*, viral competition experiments were performed. Although our CHIKV eGFP-expressing infectious clones, have similar infection properties in mosquitoes as wild-type viruses [15,35], to address potential concerns that eGFP expression might influence $OID_{50}$ values, we constructed LR-226A and LR-ApaI-226V viruses without eGFP and employed them in viral competition experiments (Figures 2A and S1). LR-ApaI-226V was derived from previously described CHIK-LR ic, by the introduction of a silent marker mutation, A6454C, in order to add an *ApaI* restriction site into the coding sequence. It was shown that the A6454C mutation does not affect the specific infectivity value (Table S1), the viral titer after RNA transfection into BHK-21 cells value (Table S1), the viral growth kinetics in BHK-21 and C6/36 cells (Figure S3), infectivity for and viral titers in *Ae. aegypti* and *Ae. albopictus* mosquitoes (Table S2), or viral fitness for growth in BHK-21 and C6/36 cells as determined by competition assay (Figure S4). These data indicate that the introduced mutation is indeed silent and does not affect the fitness of LR-ApaI-226V.

For viral competition experiments LR-ApaI-226V virus ($10^7$ plaque-forming units (pfu)) was mixed with an equal

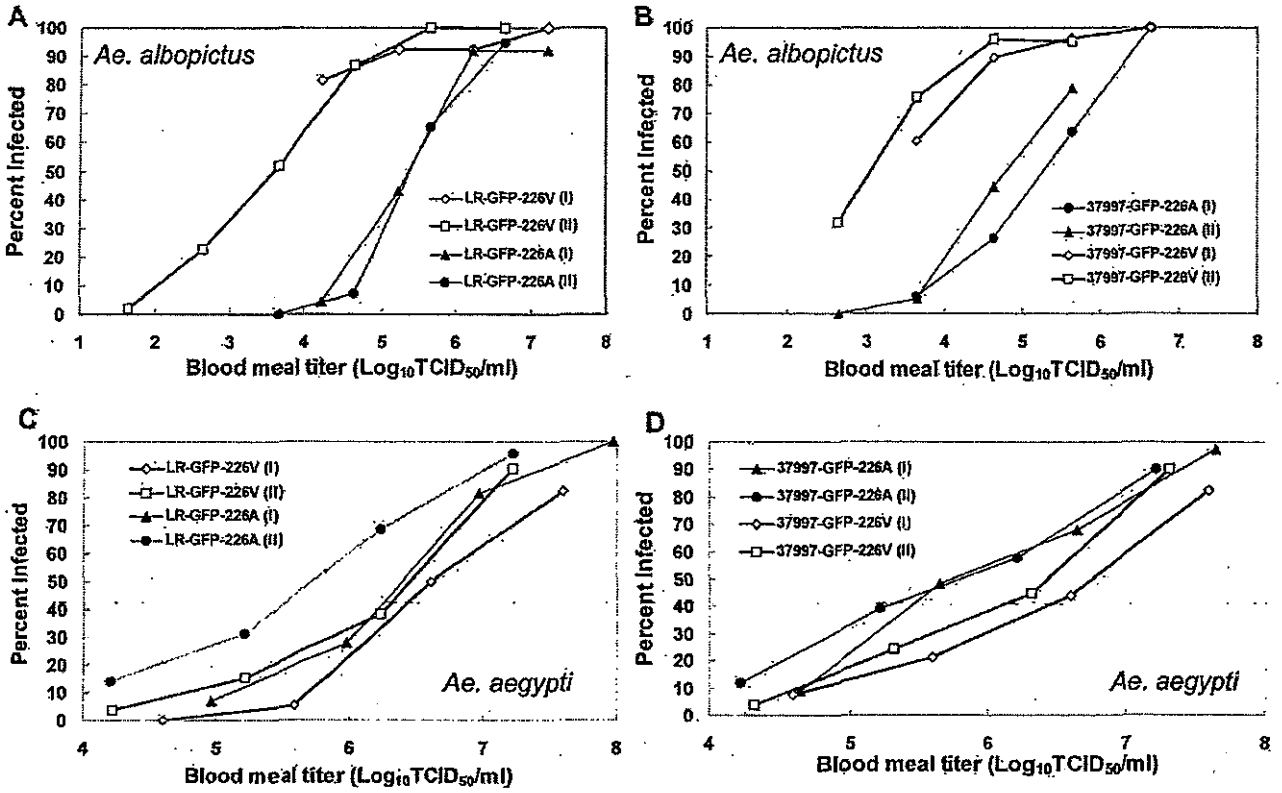



**Figure 1.** Effect of E1-A226V Mutation on CHIKV-GFP Viruses *Ae. albopictus* and *Ae. aegypti* Midgut Infectivity

Percent of orally infected *Ae. albopictus* (A, B) and *Ae. aegypti* (C, D) mosquitoes presented with blood meals containing various concentration of eGFP-expressing CHIK viruses. Serial 10-fold dilutions of viruses in the backbone of Reunion (LR-GFP-226V and LR-GFP-226A) (A, C) and 37997 (37997-GFP-226A and 37997-GFP-226V) (B, D) strains of CHIKV were made in L-15 medium followed by mixing the samples with defibrinated sheep blood. Mosquitoes were dissected at 7 dpi and eGFP expression in infected midguts was analyzed by fluorescence microscopy. A mosquito was considered infected if at least one foci of eGFP-expressing cells was present in the midgut. The experiments were performed twice for each virus (I and II).

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amount of LR-226A virus. LR-ApaI-226V and LR-226A viruses are indistinguishable in cell culture experiments (Figure S3). Mixtures of LR-ApaI-226V and LR-226A viruses were orally presented to *Ae. albopictus* mosquitoes in a blood meal, and midguts were examined at 7 dpi. The relative amount of RNA derived from LR-ApaI-226V in the midgut cells increased 5.7±0.6 times as compared to the initial relative amount of LR-ApaI-226V RNA in the blood meal sample (Figure 2B). These data support our observation that the E1-A226V mutation enhances infectivity of CHIKV for *Ae. albopictus* mosquitoes and furthermore demonstrate that the mutation could provide an evolutionary advantage over E1-226A viruses in an atypical vector and may have perpetuated the outbreak in a region where *Ae. albopictus* was the predominant anthropophilic mosquito species.

To determine if the enhanced midgut infectivity associated with the E1-A226V mutation may result in more efficient viral dissemination into secondary tissues, the kinetics of viral dissemination by LR-GFP-226V and LR-GFP-226A into salivary glands, and competition between LR-ApaI-226V and LR-226A for dissemination into mosquito heads were analyzed (Figure 3A and 3B). LR-GFP-226V virus disseminated more rapidly into *Ae. albopictus* salivary glands at all time points, with a significant difference at 7 dpi ($p=0.044$, Fisher's exact test). Similarly, in three of four replicates of competition experiments, RNA from LR-ApaI-226V virus was dramatically more abundant in the heads of *Ae. albopictus* mosquitoes as compared to RNA from LR-226A (Figure 3B, lines 1, 3, 4), although in one replica LR-ApaI-226V RNA was only slightly more abundant as compared to the initial viral RNA ratio (Figure 3B, line 2). This variability of the results may be due to random pooling of mosquito heads. Thus, replicate two may have included more heads negative for LR-

**Table 1.** $Log_{10}OID_{50}$/ml for CHIKV in *Ae. albopictus* Mosquitoes

| Backbone | Exp[a] | Virus | Mosquitoes Analyzed[b] | $Log_{10}$ $OID_{50}\pm CI_{95}$[c] | p Value |
|---|---|---|---|---|---|
| CHIK Reunion | 1 | LR-GFP-226V | 98 | <4.22 | p<0.01 |
| | | LR-GFP-226A | 101 | 5.42±0.29 | |
| | 2 | LR-GFP-226V | 171 | 3.52±0.28 | p<0.01 |
| | | LR-GFP-226A | 93 | 5.48±0.23 | |
| CHIK 37997 | 1 | 37997-GFP-226A | 131 | 5.20±0.22 | p<0.01 |
| | | 37997-GFP-226V | 138 | 3.31±0.42 | |
| | 2 | 37997-GFP-226A | 129 | 4.90±0.25 | p<0.01 |
| | | 37997-GFP-226V | 136 | 3.06±0.32 | |

$OID_{50}$ values and confidence intervals were calculated using PriProbit (version 1.63).

[a]Experiment number.

[b]Number of mosquitoes used to estimate $Log_{10}OID_{50}$/ml.

[c]95% confidence intervals.

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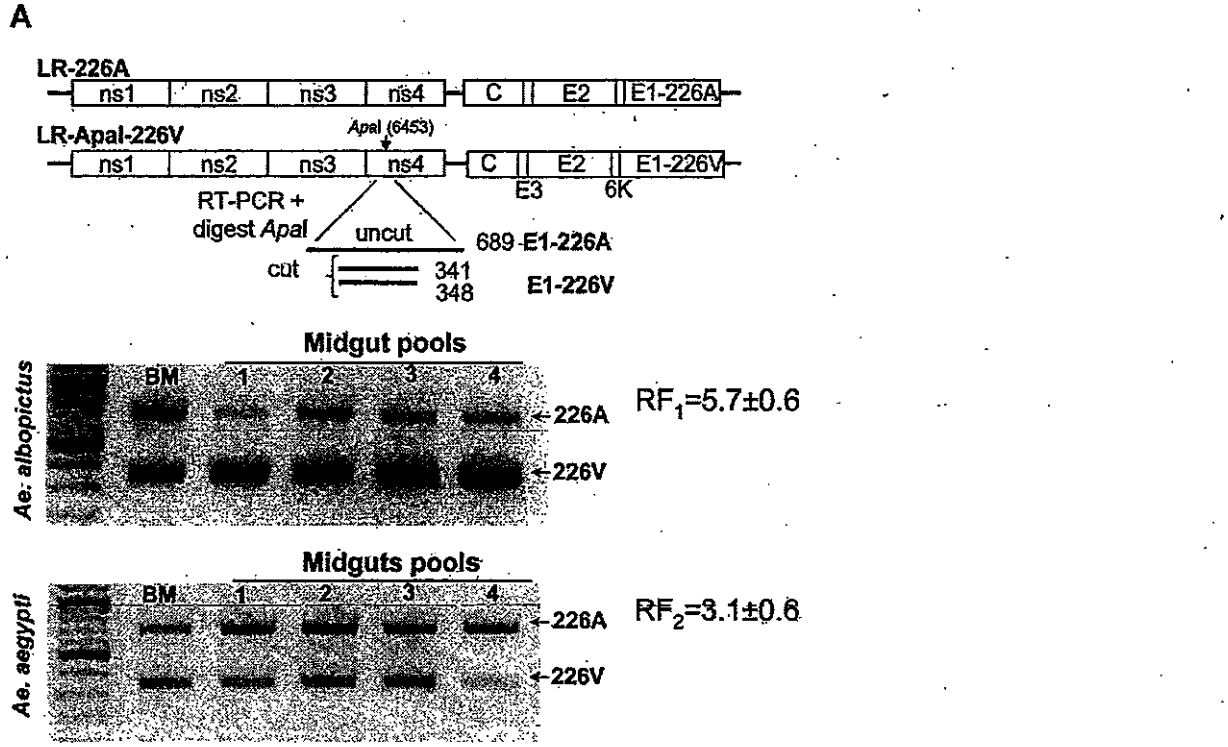


**Figure 2.** Schematic Representation of Competition Experiments (A) and Competition between LR-ApaI-226V and LR-226A Viruses for Colonization of Midgut cells of *Ae. albopictus* (B) and *Ae. aegypti* (C) Mosquitoes

$10^7$ pfu of LR-ApaI-226V and LR-226A were mixed and orally presented to *Ae. albopictus* (B) and *Ae. aegypti* (C). Viral RNAs were extracted from four pools of eight to ten midguts at 7 dpi. RT-PCR products were digested with *ApaI*, separated in 2% agarose gel, and gels were stained using ethidium bromide.

BM - initial ratio of LR-ApaI-226V and LR-226A in blood meal samples. 1–4 ratio of LR-ApaI-226V and LR-226A RNA in four independent replicas of the eight to ten midguts per replica.

Relative fitness ($RF_1$) of LR-Apa-226V to LR-226A was calculated as a ratio between 226V and 226A bands in the sample, divided by the control ratio between 226V and 226A in the blood meal.

Relative fitness ($RF_2$) of LR-226A to LR-Apa-226V was calculated as a ratio between 226A and 226V bands in the sample, divided to the control ratio between 226A and 226V in the blood meal.

Results expressed as the average of four replicas ± standard deviation (SD).

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ApaI-226V relative to heads positive for LR-226A RNA. Another possibility is that at some point during viral dissemination from the midguts into mosquito heads, LR-226A may replicate more rapidly than LR-ApaI-226V. To further investigate this relationship, *Ae. albopictus* mosquitoes were orally presented with either LR-ApaI-226V or LR-226A and whole mosquito body viral titers were compared at different time points pi. Surprisingly, no significant differences between viral titers were found, with the exception of 1 dpi, where the LR-ApaI-226V titer was 0.5 $Log_{10}$ tissues culture infectious dose 50 percent end point titer ($Log_{10}$ $TCID_{50}$/mosquito) higher than of the LR-226A titer (Figure 4A). This may be due to more efficient colonization of *Ae. albopictus* midguts by LR-ApaI-226V. The absence of significant differences in viral titers at later time points may be due to variation in viral titers among individual mosquitoes. Competition between LR-ApaI-226V and LR-226A was analyzed at different time points in order to investigate the relationship between replication of LR-ApaI-226V and LR-226A viruses in *Ae. albopictus* mosquitoes (Figure 4B). As expected, the viral RNA from LR-ApaI-226V was predominant at the early time points of 1 and 3 dpi. Interestingly, between 3 and 5 dpi the viral RNA ratio shifted toward LR-226A virus indicating that at these time points, LR-226A replicates more efficiently in some mosquito tissues (Figure 4B). This short period of time may have a slight effect on the overall outcome of competition for dissemination into salivary glands because there is a reverse shift in the RNA ratio between days 5 and 7 toward LR-ApaI-226V virus, which continues through 14 dpi. These data indicate that the E1-A226V mutation not only increases midgut infectivity but also is associated with more efficient viral dissemination from the midgut into secondary organs, suggesting that the E1-A226V mutation would increase transmissibility of CHIKV by *Ae. albopictus* mosquitoes.

A competition assay between LR-ApaI-226V and LR-226A viruses was used to examine transmission by *Ae. albopictus* to suckling mice to assess the potential for the E1-A226V mutation to influence virus transmission. *Ae. albopictus* mosquitoes were orally presented with a mixture of LR-ApaI-226V and LR-226A viruses and at 14 dpi were allowed to feed on suckling mice. Mice were sacrificed and bled on day 3 following exposure and the presence of CHIKV RNA in the blood was analyzed by RT-PCR followed by restriction digestion with *ApaI* (Figure 5B). Blood obtained from 100% of experimental mice contained detectible amounts of viral

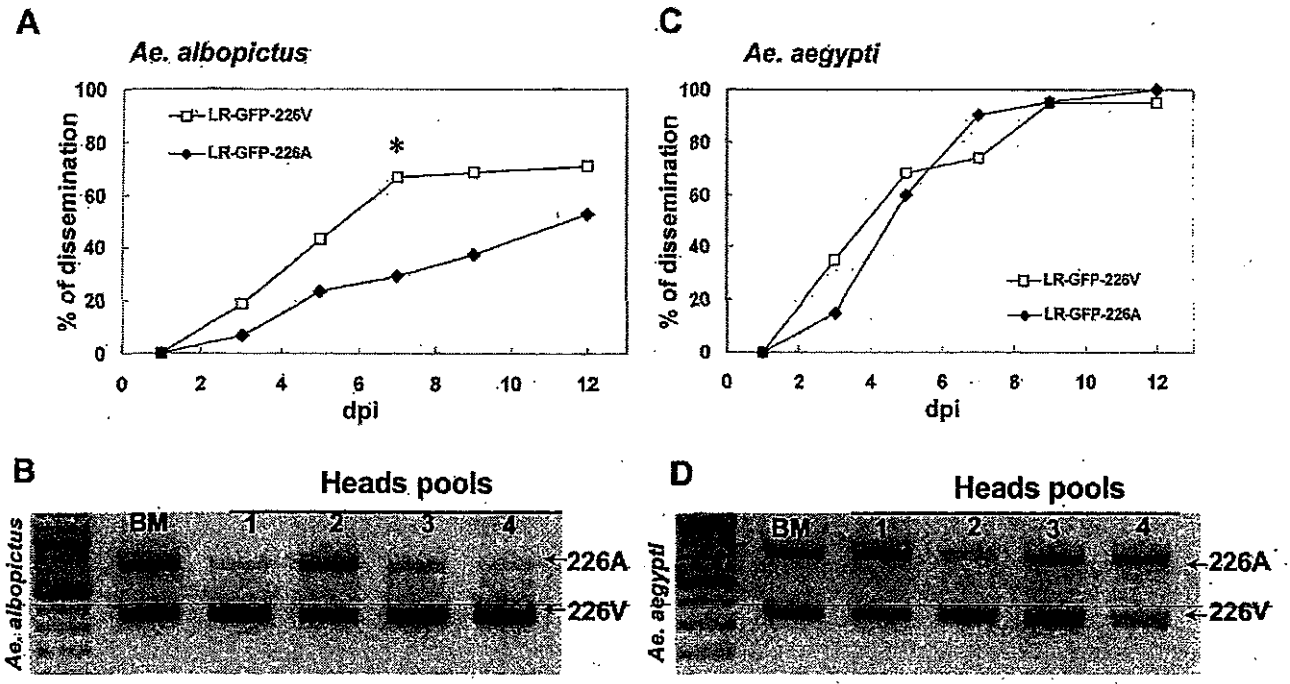


**Figure 3.** Effect of E1-A226V Mutation on CHIKV Dissemination into Salivary Glands and Heads of *Ae. albopictus* and *Ae. aegypti* Mosquitoes

*Ae. albopictus* (A) and *Ae. aegypti* (C) mosquitoes were orally infected with LR-GFP-226V and LR-GFP-226A. At the indicated time points, 16–21 mosquitoes were dissected and salivary glands were analyzed for eGFP expression. Percent of dissemination was estimated as a ratio of the number of mosquitoes with eGFP-positive salivary glands to the number of mosquitoes with eGFP-positive midguts. For *Ae. albopictus*, infectious blood meal titers were 5.95 and 6.52 $Log_{10}TCID_{50}$/ml for LR-GFP-226V and LR-GFP-226A, respectively. For *Ae. aegypti*, the infectious blood meal titer was 6.95 $Log_{10}TCID_{50}$/ml for both LR-GFP-226V and LR-GFP-226A viruses. Dissemination rates were compared statistically by Fisher's exact test using SPSS version 11.5. Asterisk indicates $p < 0.05$.

(B and D) Competition between LR-ApaI-226V and LR-226A for dissemination into heads of *Ae. albopictus* and *Ae. aegypti* mosquitoes. $10^7$ pfu of LR-ApaI-226V and LR-226A were mixed and orally presented to *Ae. albopictus* (B) and *Ae. aegypti* (D). Viral RNAs were extracted from four pools of five heads collected at 12 dpi. RT-PCR products were digested with *ApaI*, separated in 2% agarose gel, and gels were stained using ethidium bromide. BM - initial ratio of LR-ApaI-226V and LR-226A in blood meal samples. 1–4 ratio of LR-ApaI-226V and LR-226A RNA in four independent replicas of the five pooled heads per replica.

doi:10.1371/journal.ppat.0030201.g003

RNA, indicating that virus was transmitted by *Ae. albopictus* mosquitoes to suckling mice. More importantly, in all six mice analyzed, RNA derived from LR-ApaI-226V was the predominant viral RNA species, indicating that under the conditions of competition for transmission, the E1-A226V mutation directly increases CHIKV transmission by *Ae. albopictus* mosquitoes. Interestingly, in the control experiment in which mice were subcutaneously inoculated with ≈ 50 pfu of 1:1 mixture of LR-ApaI-226V and LR-226A viruses, RNAs from both viruses were readily detected and no difference was observed in the viral RNA ratio 3 dpi (Figure 5A) indicating that at least in mice, E1-A226V is not associated with changes in viral fitness.

## Effect of E1 A226V Mutation on Fitness of CHIKV in *Ae. aegypti* Mosquitoes

Since the E1-A226V mutation confers a fitness advantage in *Ae. albopictus*, it is unknown why this mutation had not been observed previously. It is possible that this change might have a deleterious effect on viral fitness in the vertebrate host, although our data of direct competition of LR-ApaI-226V and LR-226A viruses in suckling mice (Figure 5A) and analysis of CHIKV cellular tropism of four clinical isolates from Reunion (which have either A or V at position E1–226) [14], suggest that this is unlikely. An alternative hypothesis is that the E1-A226V mutation might compromise the fitness of CHIKV or have neutral fitness effects in the mosquito species which served as a vector for CHIKV prior to its emergence on Reunion island. Since *Ae. aegypti* has generally been regarded as the main vector for CHIKV prior to the emergence on Reunion island, we analyzed the effect of the E1-A226V mutation on fitness of CHIKV in *Ae. aegypti*.

In contrast to the results obtained in *Ae. albopictus* mosquitoes, $OID_{50}$ values of viruses containing the E1-226V in the backbone of the Reunion and 37997 strains of CHIKV were approximately 0.5 $Log_{10}OID_{50}$/ml higher than the $OID_{50}$ values of E1-226A viruses in all experiments using *Ae. aegypti*. These differences were statistically significant for one out of two replicates for each virus pair (Figure 1C and 1D; Table 2). A competition assay examining LR-ApaI-226V and LR-226A virus infection in *Ae. aegypti* midguts, demonstrated that LR-226A virus out-competed LR-ApaI-226V virus at 7 dpi in all four replicates using ten midguts per replicate and that the amount of LR-226A RNA increased on average 3.1 times as compared to the initial blood meal RNA ratio (Figure 2C). These data suggest that the E1-A226V mutation has a slight negative effect on CHIKV infectivity of *Ae. aegypti* midguts.

The effect of the E1-A226V mutation on the ability of CHIKV to disseminate into *Ae. aegypti* secondary organs was also analyzed (Figure 3C and 3D). LR-GFP-226V and LR-GFP-

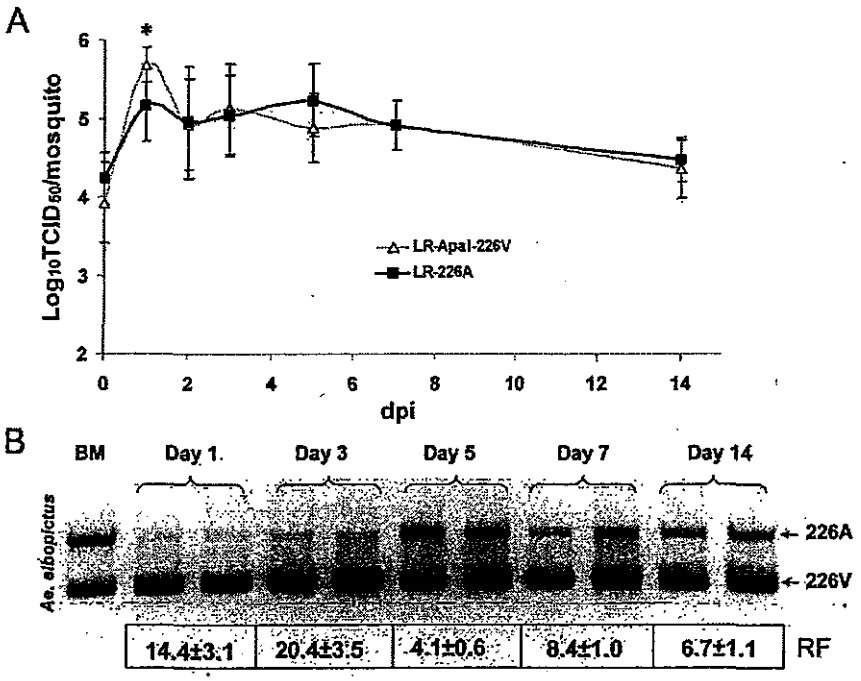


**Figure 4.** Effect of E1-A226V Mutation on CHIKV Kinetics of Viral Growth in Bodies of *Ae. albopictus* Mosquitoes

(A) Virus production in orally infected *Ae. albopictus* mosquitoes. Infected mosquitoes were sampled at 0, 1, 2, 3, 5, 7, and 14 dpi and titrated on Vero cells to estimate average titer ± standard deviation of eight whole mosquitoes. Differences in viral titers were analyzed by pairwise t-tests. Asterisk indicates $p < 0.05$.

(B) Kinetics of competition between LR-ApaI-226V and LR-226A in bodies of *Ae. albopictus* mosquitoes. $10^7$ pfu of LR-ApaI-226V and LR-226A were mixed and orally presented to *Ae. albopictus*. Infected mosquitoes were sampled at 1, 3, 5, 7, and 14 dpi. For each time point, viral RNA was extracted from two pools of ten mosquitoes.

BM - initial ratio of LR-ApaI-226V and LR-226A in blood meal samples.

RF - relative fitness of LR-Apa-226V to LR-226A was calculated as a ratio between 226V and 226A bands in the sample, divided to the control ratio between 226V and 226A in the blood meal. Results expressed as average of two replicas ± standard deviation.

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226A viruses both have similar kinetics of dissemination into salivary glands following oral infection using titers 1–2 $Log_{10}TCID_{50}$ higher than their $OID_{50}$ value in *Ae. aegypti* (Figure 3C). In a competition assay, both LR-ApaI-226V and LR-226A viruses disseminated similarly into the heads of *Ae. aegypti*. In two of four replicas, there was a slight increase in the relative amount of LR-226A RNA (Figure 3D, lines 1, 4); whereas the other two replicas showed a decrease in LR-226A RNA (Figure 2D, lines 2, 3), relative to the initial ratio of the RNA of LR-ApaI-226V and LR-226A viruses in the blood meal. A competition of LR-ApaI-226V and LR-226A viruses for transmission by *Ae. aegypti* to suckling mice was also analyzed (Figure 5C). In contrast to transmission by *Ae. albopictus* mosquitoes, five out of six mice fed upon by *Ae. aegypti* contained comparable amounts of RNA derived from both viruses and only one out of six mice contained RNA derived exclusively from LR-ApaI-226V.

### E1-A226V Mutation Modulates Cholesterol Dependence of CHIKV

It has been previously shown that a P→S mutation in the same E1–226 position of SFV releases cholesterol dependence of the virus in C6/36 cells [33] and results in significantly more rapid growth of SFV in *Ae. albopictus* mosquitoes after intrathoracic inoculation [34]. To determine if a requirement for cholesterol in the cell membrane is important for CHIKV, we analyzed cholesterol dependence of CHIKV E1-226A and E1-226V viruses (Figure 6). Growth curves of E1-226A and E1-226V viruses in the background of Indian Ocean and West African strains of CHIKV were almost indistinguishable when grown in C6/36 cells maintained in L-15 supplied with standard 10% FBS (Figure 6A). However, when the cells were depleted of cholesterol, LR-226A and 37997-226A viruses replicated significantly more rapidly than LR-226V and 37997-226V viruses, reaching 3 $Log_{10}TCID_{50}$/ml higher titer at 1, 2 and 3 dpi (Figure 6B). These data indicate that adaptation of CHIKV to *Ae. albopictus* mosquitoes coincides with CHIKV dependence on cholesterol in the target cell membrane.

## Discussion

The CHIKV outbreak in Reunion is unique because it is the first well-documented report of an alphavirus outbreak for which *Ae. albopictus* was the main vector. Interestingly, this was also the first Chikungunya epidemic during which fatal infections were reported. Our data clearly indicate that an E1-A226V mutation in CHIKV results in increased fitness of CHIKV in *Ae. albopictus* mosquitoes with respect to midgut infectivity, dissemination to the salivary glands, and transmission to a vertebrate species. These data demonstrate that a single E1-A226V mutation is sufficient to dramatically increase the ability of different strains of CHIKV to infect *Ae. albopictus* mosquitoes and that this substitution requires no

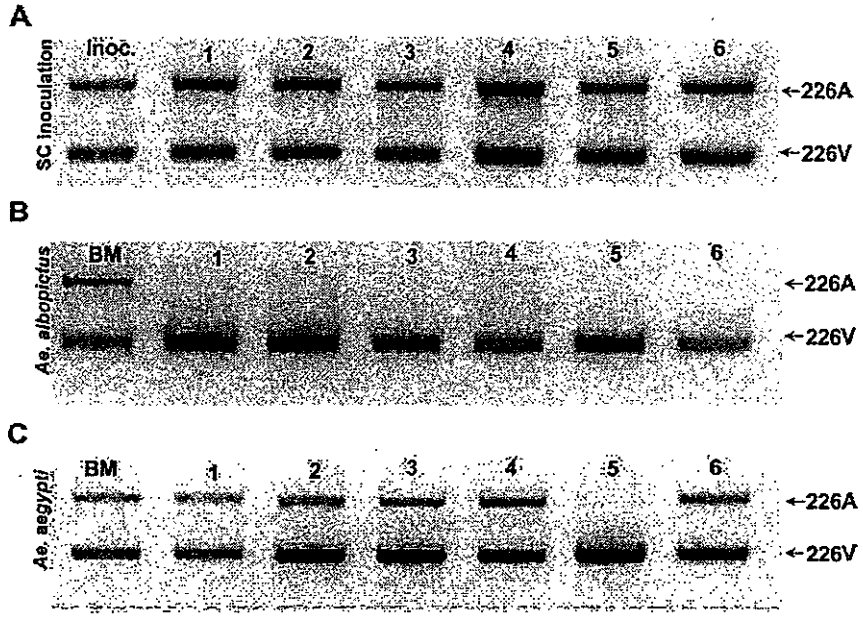


**Figure 5.** Effect of E1-A226V Mutation on CHIKV Transmission by *Ae. albopictus* and *Ae. aegypti* Mosquitoes

(A) Six 2- to 3-day-old suckling mice (Swiss Webster) were subcutaneously infected with a 20-μl mixture of ≈ 25 pfu LR-Apa-226V and ≈ 25 pfu of LR-226A viruses.

(B and C) *Ae. aegypti* and *Ae. albopictus* mosquitoes were presented with a blood meal containing $10^7$ pfu/ml of LR-Apa-226V and $10^7$ pfu/ml of LR-226A viruses. At 13 dpi, ten to 15 mosquitoes were placed in separate paper cartons and starved for 24 h. The next day, the mosquitoes in each carton were presented with a 2- to 3-day-old suckling mouse (Swiss Webster).

Mice were returned to their cage and sacrificed on day 3 post-exposure. Blood from each individual mouse (≈ 50 μl) was collected and immediately mixed with 450 μl of TRIzol reagent for RNA extraction.

BM and inoc. - initial ratio of LR-Apal-226V and LR-226A in blood meal samples and inoculum for subcutaneous infection. 1–6 ratio of LR-Apal-226V and LR-226A RNA in six individual mice.

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additional adaptive mutations to gain intermolecular compatibility. These complimentary experimental data demonstrate that a single mutation is sufficient to modify viral infectivity for a specific vector species and as a consequence, can fuel an epidemic in a region that lacks the typical vector. These observations provide the basis for an explanation of the observed rapid shift among CHIKV genotypes to viruses containing the E1-A226V mutation during the Reunion outbreak [32].

Interestingly, our data and data from previous studies [36,37] indicate that prior to acquiring the E1-A226V mutation, CHIKV is capable of producing high enough viremia in humans to efficiently infect *Ae. albopictus* mosquitoes. One explanation of the evolutionary force which allowed CHIKV to be selected so rapidly into a CHIKV strain which is adapted to *Ae. albopictus*, is that the increased infectivity (lower $OID_{50}$) of CHIKV E1-A226V mutants for *Ae. albopictus* means that the human viremic thresholds required for *Ae. albopictus* infection would likely occur earlier and be sustained for longer. Several recent studies indicate that during the course of human viremia, which last up to 6 days, CHIKV loads can reach up to $3.3x10^9$ RNA copies per ml of the blood [38,39], which corresponds to 6–7 $Log_{10}TCID_{50}$/ml [39]. Earlier studies that utilized a suckling mouse brain titration protocol, which is more sensitive than titration on Vero cells, also found that human viremia often exceeded 6 $Log_{10}SMICLD_{50}$/0.02 ml [40]. Based on viremia studies in rhesus monkeys that can develop up to 7.5 Log/ml if assayed by suckling mice brain titration [41] and a maximum viremia of only 5.5 $Log_{10}$/ml based on Vero cell titration [42], we believe that viremias in humans would correlate to 6–7 $Log_{10}TCID_{50}$/ml. From these data we calculate that the maximum virus load which can be achieved in human blood is 1–2 $Log_{10}TCID_{50}$/ml higher than the $Log_{10}OID_{50}$/ml for E1-226A viruses but 3–4 $Log_{10}TCID_{50}$/ml higher than the $Log_{10}OID_{50}$/ml for E1-226V viruses. During the course of viremia there should therefore be a substantial time frame in which CHIKV blood load is high enough for E1-226V viruses to infect *Ae. albopictus* but below the threshold for infection

**Table 2.** $Log_{10}OID_{50}$/ml for CHIKV in *Ae. aegypti* Mosquitoes

| Backbone | Exp[a] | Virus | Mosquitoes Analyzed[b] | $Log_{10}$ $OID_{50}\pm CI_{95}$[c] | p Value |
|---|---|---|---|---|---|
| CHIK Reunion | 1 | LR-GFP-226V | 65 | 6.77±0.40 | p<0.1 |
| | | LR-GFP-226A | 103 | 6.12±0.28 | |
| | 2 | LR-GFP-226V | 107 | 6.26±0.30 | p<0.05 |
| | | LR-GFP-226A | 53 | 5.62±0.33 | |
| CHIK 37997 | 1 | 37997-GFP-226A | 161 | 5.77±0.25 | p<0.01 |
| | | 37997-GFP-226V | 162 | 6.59±0.34 | |
| | 2 | 37997-GFP-226A | 136 | 5.83±0.30 | p<0.1 |
| | | 37997-GFP-226V | 127 | 6.34±0.29 | |

$OID_{50}$ values and confidence intervals were calculated using PriProbit (version 1.63).

[a]Experiment number.

[b]Number of mosquitoes used to estimate $Log_{10}OID_{50}$/ml.

[c]95% confidence intervals.

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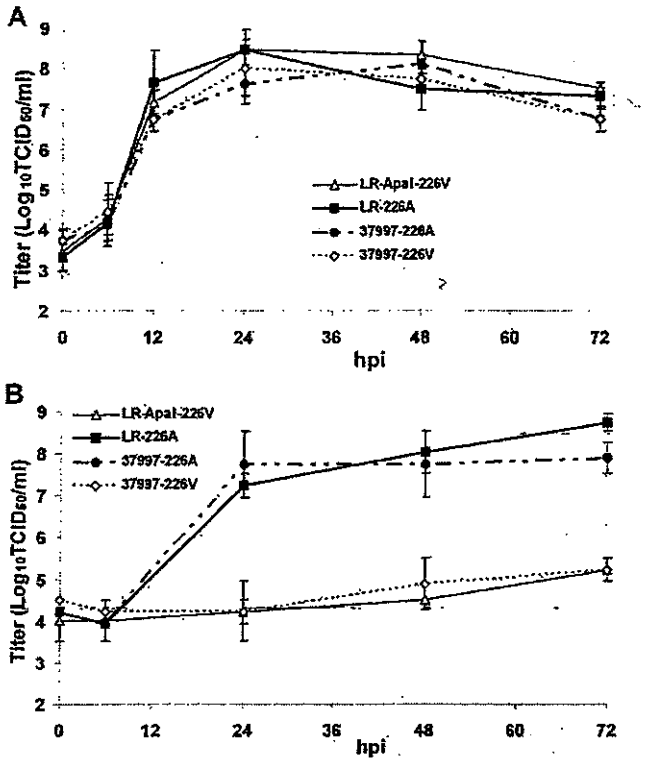


**Figure 6.** Effect of E1-A226V Mutation on *In Vitro* Growth of CHIKV in Standard (A) and Cholesterol-Depleted (B) C6/36 Cells

Cholesterol-depleted C6/36 cells were produced by five passages in L-15 medium containing 10% FBS treated with 2% CAB-O-Sil for 12 h at room temperature as previously described [52]. Confluent monolayers of standard (A) and cholesterol-depleted (B) C6/36 cells were infected with LR-ApaI-226V, LR-226A, 37997–226A and 37997–226V viruses at an MOI of 1.0 (A) and an MOI of 0.1 (B). Cells were washed three times with L-15 medium, and 5.5 ml of fresh L-15 supplied with 10% of standard or CAB-O-Sil-treated FBS were added to the flask. Cells were maintained at 28 °C. At the indicated times post-infection, 0.5 ml of medium was removed and stored at −80 °C for later titration on Vero cells. Viral titers are estimated as average $Log_{10}TCID_{50}$/ml ± standard deviation of two independent experiments.

hpi - hours post-infection.

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with E1-226A viruses. This increased opportunity for *Ae. albopictus* infection, would perpetuate the selection and transmission of the mutant virus.

During transmission competition assays, only E1-226V virus was transmitted to suckling mice by *Ae. albopictus*, although in these experiments, titers of E1-226V and E1-226A viruses were of a high enough magnitude to allow both of these viruses to efficiently infect this mosquitoes species. This indicates that there are additional mechanisms that could ensure evolutional success of the E1-A226V viruses transmitted by *Ae. albopictus*. It is possible that one of these mechanisms is associated with more efficient dissemination of the E1-226V as compared with E1-226A viruses. This could shorten the extrinsic incubation period (EIP)—the time from mosquito infection to transmission—and could have contributed to the evolutionary success of CHIKV during the Reunion outbreak because vectors infected with the LR-226V virus would transmit it more quickly than those infected with LR-226A viruses. Additionally, with relatively short-lived vectors such as mosquitoes [43], longer EIPs reduce transmission efficiency simply because fewer mosquitoes survive long enough to transmit the virus.

Our current studies do not provide data to determine if dissemination efficiency of the E1-226V viruses into the salivary glands is a consequence of more efficient midgut infectivity or if these two phenomena are independent. In this regard, it will be of particular interest to investigate the effect of the E1-A226V mutation on CHIKV transmission by orally or intrathoracically infected *Ae. albopictus* mosquitoes.

Although the CHIKV E1-A226V mutation gives a selective advantage in *Ae. albopictus*, there was not a corresponding advantage in *Ae. aegypti*. The $OID_{50}$ and midgut competition assay data indicate that E1-226V viruses were slightly less infectious for midgut cells of *Ae. aegypti* mosquitoes (Figures 1C, 1D, and 2C; Table 2). Additionally, in contrast to *Ae. albopictus*, E1-226V viruses do not have a detectable advantage for dissemination into salivary glands and heads of *Ae. aegypti*. In transmission competition experiments from *Ae aegypti* to suckling mice, E1-226V conferred a slight competitive advantage over E1-226A (Figure 5C). However, five out of six mice exposed to CHIKV infected *Ae aegypti* had equivalent amounts of both E1-226A and E1-226V viral RNAs. These results are markedly different compared to the results obtained in similar experiments using *Ae. albopictus* mosquitoes and further support the hypothesis that this E1-A226V was specifically selected as a result of adaptation of CHIKV to *Ae. albopictus* mosquitoes. To explain the small fitness advantage associated with the E1-A226V mutation which was observed in transmission experiments, we hypothesize that, similarly to *Ae. albopictus*, E1-226A and E1-226V viruses colonize different *Ae. aegypti* organs at different efficiencies. E1-226A appears to colonize midgut cells of *Ae aegypti* better than E1-226V viruses; however, following dissemination into salivary glands, the E1-226V virus gains an advantage for transmission to vertebrates.

The E1-A226V mutation was found to have a slightly negative effect on infectivity, a negligible effect on dissemination, but a slight positive effect on transmissibility of CHIKV by *Ae. aegypti* in the competition experiment. We suggest that these small (as compared with *Ae. albopictus*) differences associated with the E1-A226V mutation would not be sufficient to have a significant effect on the evolution of CHIKV transmitted by *Ae. aegypti* and would not result in accumulation of this mutation in the regions where *Ae. aegypti* serves as a primary vector for CHIKV. This may explain the lack of emergence of the E1-226V genotype in previous outbreaks and the predominance of E1-226A viruses during the 2006 CHIKV epidemic in India, in which *Ae. aegypti* is considered to be the main vector species [44]. Adaptation of African strains of CHIKV from forest dwelling mosquitoes species to *Ae. aegypti* has never been shown to be associated with any particular mutations, therefore we believe that the same negative impact of E1-A226V would be seen in African mosquito vectors which were responsible for transmission of CHIKV strains ancestral to Reunion isolates.

Our data does not exclude the possibility that the E1-A226V mutation might have a negative effect on the evolution of CHIKV transmitted by *Ae. aegypti*. Since our dissemination and transmission studies were performed using blood meal titers that were 1–2 $Log_{10}TCID_{50}$/ml higher than $Log_{10}OID_{50}$/ml values we suggest that the negative effect of decreased midgut infectivity of E1-A226V on virus trans-

missibility would be almost completely missed, simply because, under this condition, almost 100% of mosquitoes could become infected. In general, CHIKV requires significantly higher blood meal titers for infection of *Ae. aegypti* compared to *Ae. albopictus* [36,37] (Tables 1 and 2), which suggests that the slight decrease in midgut infectivity of E1-226V viruses would have a more profound effect on the evolution of CHIKV transmitted by *Ae. aegypti*, compared to the effect of a small advantage in the ability to compete with E1-226A viruses for transmission to suckling mice. Therefore, if the E1-A226V mutation occurred in CHIKV transmitted by *Ae. aegypti*, it would have a weak negative effect on viral fitness and would most likely not be preferentially selected. Additional experiments are required to evaluate this hypothesis.

Available data cannot exclude the possibility that E1-226A viruses may have an unknown beneficial effect on the fitness of CHIKV in vertebrate hosts over E1-226V viruses, and that the minor negative effect of E1-226A observed in transmission experiments by *Ae. aegypti* can be compensated for by more efficient viral replication in the vertebrate host, leading to an overall more efficient adaptation to the transmission cycle. However, comparison of the different effects of A or V residues at position E1–226 on CHIKV infectivity for, and transmission by *Ae. aegypti* and *Ae. albopictus* mosquitoes clearly suggests that polymorphisms at this position may determine the host range of the alphaviruses and may play an important role in adaptation of the viruses to a particular mosquito vector.

An interesting observation, which should be studied in more detail, was that adaptation of CHIKV to *Ae. albopictus* mosquitoes coincided with the acquisition of CHIKV dependence on cholesterol in the target membrane. It has been previously shown that various mutations in the same region of the E1 protein of SFV and Sindbis virus can modulate the cholesterol dependence of these viruses [33,45] and that SFV independence from cholesterol coincides with more rapid growth of the virus in *Ae. albopictus* [34]. Although there is an apparent association, it is currently unknown if cholesterol dependence of alphaviruses is directly responsible for modulation of fitness of alphaviruses in mosquito vectors. A possible explanation for the opposite effects of the cholesterol-dependent phenotype of SFV and CHIKV on fitness in *Ae. albopictus* may reflect the use of different techniques for mosquito infection. In our study, mosquitoes were orally infected via cholesterol rich blood meals, whereas in the previous study SFV was intrathoracically inoculated into the mosquito [34]. It is also possible that cholesterol-dependent and -independent viruses would replicate differently in different mosquito organs. As such, our data indicate that more efficient colonization of *Ae. albopictus* midgut cells by cholesterol-dependent LR-ApaI-226V is followed by relatively more rapid growth of cholesterol-independent LR –226A virus in mosquito bodies between 3 and 5 dpi (Figure 4B). Three to 5 dpi coincides with virus escape from the mosquito midgut.

Alignment of amino acid sequences that constitute the ij loop of E1 protein from different members of the alphaviruses genus revealed that position E1–226 is not conserved ([33] and data not shown) and can vary even between different strains of the same virus. In this regard, it would be reasonable to determine the cholesterol requirement of other clinically important alphaviruses, especially Venezuelan equine encephalitis virus (VEEV) and eastern equine encephalitis virus (EEEV), which show significant intra-strain variation at position E1–226 among natural isolates of these viruses, and determine mutations which can modulate their cholesterol dependence. In recent studies by Kolokoltsov et al. [46], it was suggested that VEEV, a New world alphavirus, might be cholesterol independent, although the use of Vero cells instead of C6/36 cells, and the use of different protocols for cell membrane cholesterol depletion, make it difficult to compare the results of this study with our findings. Also it would be of interest to determine possible relationships between mutations which modulate cholesterol dependence of alphaviruses other than CHIKV and on their infectivity for *Ae. aegypti* and *Ae. albopictus* mosquitoes and perhaps other epidemiologically important mosquito vectors.

The molecular mechanisms responsible for the association between host range and cholesterol dependence of CHIKV are unknown [47]. It has been proposed that upon exposure to low pH, the E1 protein of cholesterol-dependent viruses senses the target membrane lipid composition and goes through a cholesterol-dependent priming recognition reaction [48] which is not required for cholesterol-independent viruses. It is possible that CHIKV infects *Ae. aegypti* and *Ae. albopictus* midgut cells using different endocytic pathways, which targets virus to cellular compartments with different lipid contents in which fusion occurs. Specific lipids such as cholesterol may differentially affect fusion of cholesterol-dependent and cholesterol-independent CHIKV strains in these compartments and therefore define the outcome of infection. Although our observations are suggestive, more comprehensive studies should be completed to determine the exact molecular mechanisms responsible for penetration of E1-226A and E1-226V viruses into *Ae. aegypti* and *Ae. albopictus* cells.

Although previous laboratory studies have demonstrated susceptibility of *Ae. albopictus* to CHIKV infection [36,37], our data demonstrate that the E1-A226V mutation promoted infection and accelerated dissemination of CHIKV in *Ae. albopictus* mosquitoes and conferred a selective advantage over infection of *Ae. aegypti*. Whilst the mutation did not increase the maximum viral titer attainable in the mosquitoes, the synergistic effects of increased infectivity and faster dissemination of the E1-A226V virus in *Ae. albopictus* would accelerate virus transmission to a naïve human population which would have contributed to initiating and sustaining the 2005–2006 CHIKV epidemic on Reunion island. That a single amino acid change can act through multiple phenotypic effects to create an epidemic situation has implications for other arthropod-transmitted viruses and the evolution of human infectious diseases [49].

## Methods

**Viruses and plasmids.** The viruses and plasmids encoding full-length infectious clones of the LR2006 OPY1 strain CHIK-LR ic (GenBank accession number EU224268; http://www.ncbi.nlm.nih.gov/Genbank/index.html) and GFP-expressing full-length clone LR-GFP-226V (CHIK-LR 5'GFP, GenBank accession number EU224269) have been previously described [15,35]. The plasmids 37997–226A (pCHIK-37997ic, GenBank accession number EU224270) encoding full-length infectious clones of the West African strain of CHIKV 37997 and a GFP-expressing full-length clone 37997-GFP-226A (pCHIK-37997-5GFP, GenBank accession number EU224271) were derived from previously described plasmids pCHIKic and 5'CHIK EGFP [35] by

introducing CHIKV encoding cDNA into a modified pSinRep5 (Invitrogen) at positions 8055–9930. Viruses derived from 37997–226A and 37997-GFP-226A are identical to viruses derived form pCHIKic and 5'CHIK EGFP. To facilitate rapid screening of viruses in mosquitoes, the gene encoding enhance green fluorescent protein (eGFP), that is known not to compromise CHIKV phenotype in mosquitoes [15], was incorporated into clones as previously described [15]. Plasmids were constructed and propagated using conventional PCR-based cloning methods [50]. The entire PCR-generated regions of all constructs were verified by sequence analysis. The maps, sequences and detailed description of the clones are available from the authors upon request. For studies comparing the relative fitness of the mutant (E1-226V) virus and the pre-epidemic genotype (E1-226A), a silent mutation (6454C) was introduced into the CHIK-LR ic, to add an *ApaI* restriction site into the coding sequence of CHIK-LR ic. The resultant plasmid was designated LR-ApaI-226V. The E1-V226A mutation was introduced into CHIK-LR ic and LR-GFP-226V to generate plasmids designated as LR-226A and LR-GFP-226A, respectively. The mutation E1-A226V was also introduced into plasmids 37997–226A and 37997-GFP-226A. The resulted plasmids were designated 37997–226V and 37997-GFP-226V.

All plasmids were purified by centrifugation in CsCl gradients, linearized with *NotI* and *in vitro* transcribed from the minimal SP6 promoter using the mMESSAGE mMACHINE kit (Ambion) following the manufacturer's instructions. The yield and integrity of synthesized RNA were analyzed by agarose gel electrophoresis in the presence of 0.25 μg/ml of ethidium bromide. RNA (10 μg) was transfected into $1x10^7$ BHK-21 cells by electroporation as previously described [15]. Cells were transferred to 25 $cm^2$ tissue culture flasks with 10 ml of Leibovitz L-15 (L-15) medium, and supernatants were collected at 24 and 48 h post-electroporation and stored at −80 °C. In parallel, $1x10^5$ electroporated BHK-21 cells were serially 10-fold diluted and seeded in six-well plates for infectious centers assay as previously described [15].

**Cells and mosquitoes.** BHK-21 (baby hamster kidney) cells were maintained at 37 °C in L-15 medium supplemented with 10% fetal bovine serum (FBS), 100 U penicillin, and 100 μg/ml streptomycin. C6/36 cells (*Ae. albopictus*) were grown in the same medium at 28 °C. *Ae. aegypti* (white-eyed Higgs variant of the Rexville D strain) and *Ae. albopictus* (Galveston strain) were reared at 27 °C and 80% relative humidity under a 16h light: 8h dark photoperiod, as previously described [35]. Adults were kept in paper cartons supplied with 10% sucrose on cotton balls. To promote egg production females were fed on anaesthetized hamsters once per week.

Rexville D strain of *Ae. aegypti* mosquitoes were originally selected for susceptibility to flavivirus infection [51]. Since there are no known consequences of this original selection with respect to susceptibility to CHIKV, a white eyed variant of the strain that facilitates detection of GFP was used in our experiments.

***In vitro* virus growth of CHIKV in standard and cholesterol-depleted C6/36 cells.** To investigate if the mutation influenced cholesterol dependence of the virus, cholesterol-depleted C6/36 cells were prepared by five passages in L-15 medium containing 10% FBS treated with 2% CAB-O-Sil (Acros Organics) for 12 h at room temperature as previously described [52]. CHIKV growth curves were determined by infecting cholesterol-depleted and normal C6/36 cells at a multiplicity of infection (MOI) of 0.1 and 1.0, respectively, by rocking for 1 h at 25 °C. The cells were washed three times with L-15 medium and 5.5 ml of fresh L-15 supplied with 10% of standard or CAB-O-Sil treated FBS was added to the flask. At the indicated times post-infection, 0.5 ml of medium was removed and stored at −80 °C until titrated. The volume of medium was then restored by adding 0.5 ml of appropriate medium.

**Titrations.** Viral titers from mosquito samples and from tissue culture supernatant were determined using Vero cells and expressed as tissue culture infectious dose 50 percent endpoint titers ($Log_{10}TCID_{50}$) as previously described [53]. Additionally, for viral competition experiments, titers of LR-Apa-226V LR-226A viruses were determined using standard plaque assay on Vero cells as previously described [54].

**Oral infection of mosquitoes.** *Ae. aegypti* and *Ae. albopictus* were infected in an Arthropod Containment Level 3 insectary as described previously [35,55]. To make infectious blood meals for the viruses lacking eGFP, viral stocks derived from electroporated BHK-21 cells were mixed with an equal volume of defibrinated sheep blood and supplemented with 3 mM ATP as a phago-stimulant. To produce infectious blood meals for the eGFP-expressing viruses, the viruses were additionally passed on BHK-21 cells. The cells were infected at a MOI ≈ 1.0 with virus derived from electroporation. At 2 dpi, cell culture supernatants were mixed with an equal volume of defibrinated sheep blood and presented to 4- to 5-day-old female mosquitoes that had been starved for 24 h, using a Hemotek membrane feeding system (Discovery Workshops) and hamster skin membrane. Mosquitoes were allowed to feed for 45 min, and engorged mosquitoes (stage ≥3+ [56]) were sorted and returned to a cage for maintenance. Blood meals and three to four mosquitoes were immediately removed for titration and/or RNA extraction. Depending on the purpose of the experiments, mosquitoes were collected at different days post-infection and either titrated to determine viral titer, dissected for analysis of eGFP expression in the midguts or salivary glands [15], or used for RNA extraction in competition experiments.

To estimate the Oral Infectious Dose 50% values ($OID_{50}$), serial 10-fold dilutions of viruses were made in L-15 medium followed by mixing the samples with defibrinated sheep blood. Mosquitoes were dissected at 7 dpi and eGFP expression in infected midguts was analyzed by fluorescence microscopy. A mosquito was considered infected if at least one foci of eGFP-expressing cells was present in the midgut. The experiments were performed twice for each virus. $OID_{50}$ values and confidence intervals were calculated using PriProbit (version 1.63).

**Viral competition experiments.** To test the hypothesis that the E1-A226V mutation might be associated with a competitive advantage in mosquito vectors, competition assays were designed similar to those described previously in mice [57], with minor modifications (Figure 2A). Both *Ae. aegypti* and *Ae. albopictus* mosquitoes were presented with a blood meal containing $10^7$ plaque-forming units (pfu)/ml of LR-Apa-226V and $10^7$ pfu/ml of LR-226A viruses. It had been previously found that for these two viruses the ratio of viral RNAs corresponds to the ratio of viral titers (data not shown). Midguts were collected at 7 dpi and analyzed in pools of eight to ten, and heads were collected at 12 dpi and analyzed in pools of five. RNA was extracted from the tissue pools using TRIzol reagent (Invitrogen) followed by additional purification using a Viral RNA mini kit (QIAGEN). RNAs from blood meal samples were extracted using Viral RNA Mini Kit followed by treatment with DNAse (Ambion) to destroy any residual plasmid DNA contaminant in the viral samples. RNA was reversed transcribed from random hexamer primers using Superscript III (Invitrogen) according to the manufacturer's instructions. cDNA was amplified from 41855ns-F5 (5'- ATATCTAGACATGGTGGAC) and 41855ns-R1 (5'-TATCAAAGGAGGCTATGTC) primers using Taq DNA polymerase (New England Biolabs). PCR products were purified using Zymo clean columns (Zymo Research) and were quantified by spectrophotometry. Equal amount of PCR products were digested with *ApaI*, separated in 2% agarose gels that were stained using ethidium bromide. Thus the LR-Apa-226V and LR-226A viruses could be distinguished by size on an agarose gel (Figure 2A). Gel images were analyzed using TolaLab (version 2.01). Relative fitness of LR-Apa-226V and LR-226A viruses was calculated as a ratio between 226V and 226A bands in the sample, divided by the control ratio of 226V and 226A in the blood meal.

**Virus competition in an animal transmission model.** *Ae. aegypti* and *Ae. albopictus* mosquitoes were presented with a blood meal containing $10^7$ pfu/ml of LR-Apa-226V and $10^7$ pfu/ml of LR-226A viruses. At 13 dpi, ten to 15 mosquitoes were placed in separate paper cartons and starved for 24 h. The next day the mosquitoes in each carton were presented with individual 2- to 3-day-old suckling mouse (Swiss Webster). Feeding continued until 2–3 mosquitoes per carton were fully engorged (stage ≥3+[56]). In a parallel experiment six 2- to 3-day-old suckling mice were subcutaneously infected with 20 μl of mixture containing ≈ 25 pfu of LR-Apa-226V and ≈ 25 pfu of LR-226A viruses. Mice were returned to their cage and sacrificed on day 3 post-exposure. Blood from each individual mouse (≈ 50 μl) was collected and immediately mixed with 450 μl of TRIzol reagent for RNA extraction. The RNA was processed as described above. All animal manipulations were conducted in accordance with federal laws, regulations, and in compliance with National Institutes of Health and University of Texas Medical Branch Institutional Animal Care and Use Committee guidelines and with the Association for Assessment and Accreditation of Laboratory Animal Care standards.

## Supporting Information

**Figure S1.** Schematic Representation of the Viruses Used in This Study

Found at doi:10.1371/journal.ppat.0030201.sg001 (917 KB PDF).

**Figure S2.** Growth of the eGFP-Expressing Viruses in BHK-21(A, C) and C6/36 (B, D) Cells

Confluent monolayers of BHK-21 and C6/36 cells in T25 tissue culture flacks were infected with LR-GFP-226V and LR-GFP-226A (A,

B) or 37997-GFP-226A and 37997-GFP-226V viruses derived from electroporation at a MOI of 0.1. At the indicated times post-infection, 0.5 ml of medium was removed and stored at −80 °C for later titration on Vero cells. Viral titers are expressed as $Log_{10}TCID_{50}$/ml.

Found at doi:10.1371/journal.ppat.0030201.sg002 (372 KB PDF).

**Figure S3.** Growth of the CHIK-LR ic, LR-ApaI-226V and LR-226A Viruses in BHK-21(A) and C6/36 (B) Cells

Confluent monolayers of BHK-21 and C6/36 cells in T25 tissue culture flacks were infected with LR-GFP-226V and LR-GFP-226A (A, B) or 37997-GFP-226A and 37997-GFP-226V viruses derived from electroporation at a MOI of 1.0. At the indicated times post-infection, 0.5 ml of medium was removed and stored at −80 °C until titrated on Vero cells. Viral titers are expressed as $Log_{10}TCID_{50}$/ml ± standard deviation of three independent experiments.
hpi - hours post-infection.

Found at doi:10.1371/journal.ppat.0030201.sg003 (177 KB PDF).

**Figure S4.** Competition between CHIK-LR ic and LR-ApaI-226V for Growth in BHK-21 and C6/36 Cells

Cells were infected with a 1:1 mixture of both viruses at a MOI of 0.001. 2 dpi, cell culture supernatant was collected and samples proceeded as described. The experiment was repeated three times for each of the cell types.
inoc - initial ratio of CHIK-LR ic and LR-ApaI-226V in the inoculum used for infection of cells.
Relative fitness (RF) of CHIK-LR ic and LR-ApaI-226V was calculated as an average ratio between CHIK-LR ic and LR-ApaI-226V bands in the supernatant obtained from BHK-21 cells ($RF_1$) and C6/36 cells ($RF_2$), divided by the control ratio between CHIK-LR ic and LR-ApaI-226V in the inoculum.

Found at doi:10.1371/journal.ppat.0030201.sg004 (3.6 MB PDF).

**Table S1.** Specific Infectivity and Virus Titers after Electroporation

a - amino acids at position of E1–226.
b - Specific infectivity of *in vitro* transcribed RNA. $10^7$ BHK-21 cells were transfected with 10 μg of RNA. Electroporated BHK-21 cells were 10-fold serially diluted, seeded in 6-well tissue culture plates containing $5x10^5$ naive BHK-21 cells per well and covered with 0.5% agarose in L-15. Plaques were scored on day 2 post-transfection.
c - Supernatants of electroporated BHK-21 cells were collected on days 1 and 2. Virus titers were determined by titration on Vero cells and expressed as $Log_{10}TCID_{50}$/ml.
hpi - hours post-infection.

Found at doi:10.1371/journal.ppat.0030201.st001 (34 KB DOC).

**Table S2.** Infection Rates and Average Titers of CHIKV-LR ic or LR-ApaI-226V in Orally Infected *Ae. aegypti* and *Ae. albopictus*

*Ae. aegypti* mosquitoes were orally presented with 7.24±0.4 $Log_{10}TCID_{50}$/ml of CHIKV-LR ic (summary of two experiments) and 6.52 $Log_{10}TCID_{50}$/ml of LR-ApaI-226V.
*Ae. albopictus* mosquitoes were orally presented with 7.24±0.4 $Log_{10}TCID_{50}$/ml of CHIKV-LR ic (summary of two experiments) and 7.52 $Log_{10}TCID_{50}$/ml LR-ApaI-226V.
At 7 and 14 dpi, mosquitoes were collected and triturated in 1mL of L-15 medium for titration on Vero cells.
Titers are reported as $Log_{10}TCID_{50}$/ml ± standard deviation.

Found at doi:10.1371/journal.ppat.0030201.st002 (31 KB DOC).

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**Author contributions.** KAT and SH conceived and designed the experiments. KAT, DLV, and CEM performed the experiments and analyzed the data. KAT, DLV, CEM, and SH wrote the paper.

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**Competing interests.** The authors have declared that no competing interests exist.

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# 医薬品 研究報告 調査報告書

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| 販売名(企業名) | 赤血球濃厚液-LR「日赤」(日本赤十字社)<br>照射赤血球濃厚液-LR「日赤」(日本赤十字社) | | | [1]中国[2]ベトナム[3]パキスタン[4]汎米保健機構 |

**研究報告の概要**

○デングウイルス最新情報

[1]中国(福建省):9月30日、保健当局は福建省莆田市でデング熱症例39例を確認したと発表した。感染拡大を防ぐ為の総合的予防対策が実施され、医療機関でのモニタリングが強化されている。市民には、蚊の増殖を防ぐ為に衛生状態改善が呼びかけられている。

[2]ベトナム:2007年のデング熱発生件数は、昨年と比べて50%増加したと保健省が報告した。9月24日時点で患者68,000人が報告され、60人が死亡した。感染例のほとんどは南部で発生している。患者は通常10歳以下の子どもが多いが、2007年は成人患者も増加している。

[3]パキスタン(カラチ):保健省のデング熱サーベイランス班によると、カラチ市の4つの病院で22例の新規デング熱疑い症例が報告された。うち20人が陽性、2人が検査中となっている。2007年はこれまでに170例の疑い症例が報告された。

[4]ラテンアメリカ:デング熱がラテンアメリカとカリブ海諸国に感染拡大しており、この10年で最も深刻な事態になっている。2007年はこれまでに630,356人の患者が主にブラジル・ベネズエラ・コロンビアから報告され、うち12,147人が出血熱を発症、183人が死亡した。このまま拡大が続けば2002年の1,015,000例を超える可能性がある。流行が沈静化しないと社会的、経済的に大きな影響が出るだろうと汎米保健機構の専門家は述べている。観光や移住によって4系統のウイルス株が地域内で循環しているために、患者が重症化しやすくなっていると考えられている。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 中国福建省、ベトナム、パキスタン、ラテンアメリカでデング熱が流行しているとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。問診でデング熱の既往があった場合には、治癒後1ヶ月間献血不可としている。今後も引き続き情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

Back

**Archive Number** 20071001.3237
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**Subject** PRO/EDR> Dengue/DHF update 2007 (37)

DENGUE/DHF UPDATE 2007 (37)
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*In this update:*
[1] China (Fujian)
[2] Viet Nam
[3] Pakistan (Karachi)
[4] Latin America

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[1] China (Fujian)
Date: Sun 30 Sep 2007
Source: China Daily, Xinhua News Agency report [edited]
<http://www.chinadaily.com.cn/china/2007-09/30/content_6149071.htm>

On Sunday [30 Sep 2007], health authorities said 39 dengue fever cases have been confirmed in Putian City of east China's Fujian Province. Thus far, 26 of the 39 patients in Hanjiang District of Putian City have been cured and the others are in stable condition, said the provincial health department.

The city has adopted "comprehensive prevention and control measures" to curb the spread of the disease, said the department. All medical and health institutions in the province have also strengthened monitoring on the disease, it added.

The department reminded citizens of household sanitation and the prevention of proliferation of mosquitoes, which transmit the disease [v as]

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Communicated by:
ProMED-mail Rapporteur Brent Barrett

[Putian City is situated in the central part of the coastal area of Fujian Province. Putian neighbors Fuzhou in the northeast and Quanzhou in the southeast, and is separated from Taiwan by the Taiwan Strait.

[A zoomable map of Fujian Province showing the location of Putian city can be accessed at
<http://encarta.msn.com/map_701510630/Fujian.html>. - Mod.TY]

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[2] Viet Nam
*Date: Wed 26 Sep 2007*
Source: VietNamNet Bridge [edited]
<http://english.vietnamnet.vn/social/2007/09/745035/>

The incidence of dengue fever in Viet Nam has risen by almost 50 percent this year [2007] against last year [2006], reports the Health Minist

A medical worker instructs Dao ethnic minorities in the northern mountainous province of Yen Bai's Quang Minh Commune to dip mosquito

nets in chemicals to prevent dengue fever.

About 68 000 people had been stricken with the mosquito-borne disease, Preventative Health Department director Nguyen Huy Nga said on Monday [24 Sep 2007]; 60 had died.

Most infections had occurred in southern Dong Thap, An Giang, Tien Giang, and Ben Tre provinces and the total increase was about 48 percent, he said.

Ho Chi Minh [HCM] City-based Pasteur Institute National Dengue Fever Programme representative Luong Chan Quang said more than 58 000 people had been infected in the Cuu Long (Mekong) Delta provinces by the end of August [2007]. Deaths were put at 54-40 percent more than last year [2006].

Infections in Tien Giang Province totalled 9800 with 9 deaths, Dong Thap 8700 with 9 deaths, and An Giang 6000 with 6 deaths.

In HCM City, almost 5400 people had been stricken with dengue fever -- 40 percent more than last year [2006 -- and 6 had died.

Quang warned that another serious outbreak was likely in the southern delta before the end of the year [2007] if effective preventive measures were not taken because people regularly stored water to prepare for the dry season.

The _Aedes_ mosquito, which carries dengue fever, breeds in still or stagnant water.

HCM City Preventive Health Department deputy director Nguyen Dac Tho said about 350 people were being admitted to hospital each week with dengue fever -- 50 more than last year [2006].

Inner city districts 8, 10, 11, Binh Thanh, and Binh Tan were the worst affected. People in densely populated precincts stored more water for their own use than others as did construction projects, said Dr Tho.

Dengue fever is most common among children under 10 but the number of afflicted adults has increased this year [2007].

HCM City Tropical Diseases Hospital figures show that of about 150 people admitted to the hospital with dengue fever each week, more than 100 were adults.

There are 4 types of the dengue fever virus that often result in similar symptoms. This year [2007], the transmitted virus was usually type 1 or type 2.

Haemorrhagic fever is a severe, often fatal, complication of dengue fever.

The HCM City People's Committee has mobilised measures to prevent dengue fever across the city. Citizens are encouraged to clean around their residences every Sunday and spray mosquito killer [insecticides].

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<promed@promedmail.org>

[A map of Viet Nam can be accessed at
<http://www.lib.utexas.edu/maps/middle_east_and_asia/vietnam_admin01.jpg>.
- Mod.TY]

******
[3] Pakistan (Karachi)
Date: Thu 27 Sep 2007
Source: Daily Times [edited]
<http://www.dailytimes.com.pk/default.asp?page=2007%5C09%5C27%5Cstory_27-9-200

The Sindh Health Department's Dengue Fever Surveillance Cell reported 22 fresh cases of the disease in select hospitals across Karachi on Wednesday [26 Sep 2007].

Additional health secretary and in-charge of the surveillance cell, Dr Shakil Malik, giving details of these cases, told APP [Associated Press of Pakistan] that 20 of the patients are positive and they are waiting for the report on the other 2.

The hospitals that dispatched reports include Liaquat National Hospital, Ziauddin Hospital, Bismillah Taqee Hospital, and Zainab Panjwani Hospital. "Since we just reactivated the cell on Tuesday [25 Sep 2007], it will take time before we make contact with all the hospitals scattered across the city," he said. To a question, he said that around 170 suspected cases of dengue fever have been reported from across the city this year -- from January [2007] to date. He also referred to the report the provincial health department received from a local laboratory (Mid Citi Lab) that tested 24 OPD [out patient department] patients between August [2007] and now. Of these individuals, 12 came out positive.

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Communicated by:
ProMED Rapporteur Brent Barrett

[Karachi is located on the Arabian Sea. A map of Pakistan can be accessed at <http://www.lib.utexas.edu/maps/middle_east_and_asia/pakistan_pol_2002.jpg>. - Mod.TY]

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[4] Latin America
Date: Sat 29 Sep 2007
Source: Associated Press [edited]
<http://ap.google.com/article/ALeqM5i86GcnUASvmXnPi9bBXcqngijdSQD8RVA16O4>

Dengue fever is spreading across Latin America and the Caribbean in one of the worst outbreaks in decades, causing agonizing joint pain for hundreds of thousands of people and killing nearly 200 so far this year [2007].

The mosquitoes that carry dengue are thriving in expanded urban slums scattered with water-collecting trash and old tires. Experts say dengue is approaching record levels this year [2007] as many countries enter their wettest months.

"If we do not slow it down, it will intensify and take a greater social and economic toll on these countries," said Dr. Jose Luis San Martin, head of anti-dengue efforts for the Pan American Health Organization (PAHO), a regional public health agency.

The US Centers for Disease Control and Prevention (CDC) in Atlanta has posted advisories this year [2007] for people visiting Latin American and Caribbean destinations to use mosquito repellant and stay inside screened areas whenever possible.

"The danger is that the doctors at home don't recognize the dengue," said Dr. Wellington Sun, the chief of the CDC's dengue branch in San Juan, [Puerto Rico] "The doctors need to raise their level of suspicion for any traveler who returns with a fever."

Dengue has already damaged the economies of countries across the region by driving away tourists, according to a document prepared for a PAHO conference beginning Monday [1 Oct 2007] in Washington.

Some countries have focused mosquito eradication efforts on areas popular with tourists. Mexico sent hundreds of workers to the resorts of Puerto Vallarta, Cancun, and Acapulco this year [2007] to try to avert outbreaks.

Health ministers from across the region meet at the PAHO conference and San Martin said he will urge them to devote more resources to dengue feve

The tropical virus was once thought to have been nearly eliminated from Latin America, but it has steadily gained strength since the early 1980s. Now, officials fear it could emerge as a pandemic similar to one that became a leading killer of children in Southeast Asia following World War II.

Officials say the virus is likely to grow deadlier in part because tourism and migration are circulating 4 different strains across the region. A person exposed to one strain may develop immunity to that strain -- but subsequent exposure to another strain makes it more likely the person will develop the hemorrhagic form.

"The main concern is what's happening in the Americas will recapitulate what has happened in Southeast Asia, and we will start seeing more and more severe types of cases of dengue as time progresses," Sun said.

So far this year [2007], 630 356 dengue cases have been reported in the Americas -- most in Brazil, Venezuela, or Colombia -- with 12 147 cases of hemorrhagic fever and 183 deaths, according to the Pan American Health Organization. With the spread expected to accelerate during the upcoming rainy season in many countries, cases this year [2007] could exceed the 1 015 000 reported in 2002, according to San Martin.

In Puerto Rico, where 5592 suspected cases and 3 deaths have been reported, some lawmakers called this week for the health secretary to resign.

In the Dominican Republic, which has reported 25 deaths this year [2007], the health department announced Thursday [27 Sep 2007] that it would train 2.5 million public school students to encourage parents and neighbors to eliminate standing water.

Researchers have not yet developed a vaccine against dengue and Sun said that for now, the only way to stop the virus is to contain the mosquito population -- a task that relies of countless, relentless individual efforts including installing screen doors and making sure mosquitoes are not breeding in garbage.

"It's like telling people to stop smoking," he said. "They may do it for a while, but they don't do it on a consistent basis and without doing that, it's not effective."

While dengue is increasing around the developing world, the problem is most dramatic in the Americas, according to the CDC.

Health officials believe the resurgence of the malaria-like illness is due partly to a premature easing of eradication programs in the 1970s.

Migration and tourism also have carried new strains of the virus across national borders, even into the United States, which had largely wiped out the disease after a 1922 outbreak that infected a half-million people.

Mexico has been struggling with an alarming increase in the deadly hemorrhagic form of dengue, which now accounts for roughly one in 4 cases. The government has confirmed 3249 cases of hemorrhagic dengue for the year through 15 Sep [2007], up from 1924 last year [2006].

The CDC says there is no drug to treat hemorrhagic dengue, but proper treatment, including rest, fluids, and pain relief, can reduce death rates to about one percent.

San Martin said he use the meetings starting Monday [1 Oct 2007] to urge enforcement of trash disposal regulations, more investment in mosquito control and new incentives for communities to participate. "It is a battle of every government, every community and every individual," he said.

[Byline: Michael Melia]

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ProMED-mail
<promed@promedmail.org>

The WHO (World Health Organisation) description of dengue fever and the more deadly dengue hemorrhagic fever [DHF] can be found in ProMED-mail's "Dengue/DHF update 20070514.1541". ProMED-mail thanks the contributors to this update and encourages others to contribute reports also. - Mod.TY] 97

[see also:
Dengue/DHF update 2007 (36) 20070924.3165
Dengue/DHF update 2007 (35) 20070918.3103
Dengue/DHF update 2007 (34) 20070908.2964
Dengue/DHF update 2007 (33) 20070821.2726
Dengue/DHF update 2007 (32) 20070816.2675
Dengue/DHF update 2007 (31) 20070806.2555
Dengue/DHF update 2007 (30) 20070730.2440
Dengue/DHF update 2007 (20) 20070514.1541
Dengue/DHF update 2007 (10) 20070225.0683
Dengue/DHF update 2007 (01) 20070103.0030]
.....ty/mj/mpp

別紙様式第2-1

No. 34

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 10. 16 | 該当なし | |

| 一般的名称 | 人赤血球濃厚液 | 研究報告の公表状況 | | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 赤血球濃厚液-LR「日赤」(日本赤十字社)<br>照射赤血球濃厚液-LR「日赤」(日本赤十字社) | | Yahoo!ニュース. 2007 Oct 14. | 台湾 |

**研究報告の概要**

○台南で511人が感染！猛威をふるうデング熱、「蚊」撲滅作戦に軍も動員へ―台湾南部
台湾のニュースサイト「中国台湾網」などが伝えたところによると、台湾南部でデング熱が流行。台南市政府の最新の調査では、2007年10月13日までに市内で511人の感染者が確認されたほか、隣接する高雄市でも2つの区で集団感染が発生しており、感染の広がりは過去最大規模。
高雄市では来週、スポーツ競技大会が予定されており、選手団の感染を防ぐため競技会場と選手村周辺地域を警戒重点区域に指定した。市職員のほか、軍も動員し、デング熱ウイルスを媒介する蚊の撲滅作戦を展開する方針だ。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 台湾南部でデング熱が流行し、台南市内で511人の感染者が確認されたほか、隣接する高雄市でも集団感染が発生しており、感染の広がりは過去最大規模となっているとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。問診でデング熱の既往があった場合には、治癒後1ヶ月間献血不可としている。今後も引き続き情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

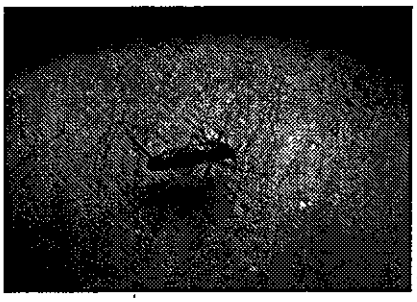
(17)

海外



# 台南で511人が感染！猛威をふるうデング熱、「蚊」撲滅作戦に軍も動員へ—台湾南部

10月14日9時38分配信 Record China



拡大写真

台湾南部で蚊が媒介するデング熱が大流行。10月13日までに台南市で511人の感染が確認されたほか、高雄市でも集団感染が発生。行政と軍が協力して大規模な蚊の撲滅作戦を展開する方針。

台湾のニュースサイト「中国台湾網」などが伝えたところによると、台湾南部でデング熱が流行。台南市政府の最新の調査では、2007年10月13日までに市内で511人の感染者が確認されたほか、隣接する高雄市でも2つの区で集団感染が発生しており、感染の広がりは過去最大規模。

高雄市では来週、スポーツ競技大会が予定されており、選手団の感染を防ぐため競技会場と選手村周辺地域を警戒重点区域に指定した。市職員のほか、軍も動員し、デング熱ウイルスを媒介する蚊の撲滅作戦を展開する方針だ。（翻訳・編集/本郷智子）

最終更新:10月14日9時38分

Record China

別紙様式第2-1

No. 11

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 10. 22 | 該当なし | |

| 一般的名称 | 人赤血球濃厚液 | 研究報告の公表状況 | | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 赤血球濃厚液-LR「日赤」(日本赤十字社)<br>照射赤血球濃厚液-LR「日赤」(日本赤十字社) | | 三浦左千夫, 肥後廣夫, 竹内勤.<br>第48回日本熱帯医学会大会 | 日本 |

**研究報告の概要**

○日本におけるラテンアメリカ人の慢性シャーガス病キャリアーからの献血についての対策検討

近年ラテンアメリカからの就労目的の定住化人口が増加の一途にあり、既に40万人を超えようとしている。当然就労目的のため、表向きは健常者としての来日である。しかし、就労中に疲れを訴え呼吸困難などの不調を来たした為に医療機関を受診し、出身地を考慮の後、血清免疫学的検査の結果シャーガス病感染を示唆された者が13名見いだされた。中には、末梢血で病原体*Trypanosoma cruzi*(*T. cruzi*)の存在を示唆する*T. cruzi* DNAのPCR増幅断片が、検査の度に検出される者もいた。また血液培養(LIT培地、NNN培地)で血液型虫体の分離にも成功した例など、慢性シャーガス病即ち病原体のキャリアーであることが明らかとなった者もいた。これらの抗体陽性者13名のうち1名については日本滞在中に献血を行っていた。注意すべきは、我が国では主要媒介昆虫が棲息しないものの、シャーガス病慢性キャリアーからの輸血、臓器移植などによる二次的感染の危険性である。今回、献血機関で保存血用に使用しているカーミC液(CPD液)を用いて感染マウス血液を4℃にて1～21日間保存処理を行った。これを正常マウスに接種し感染性、病原性について基礎的な検討を行った。その結果マウスへの感染性は無処置のものと差異は無かったが、病原性についてはかなり減弱していることが示唆された。これは*T. cruzi*に対して4℃という低温ストレスが影響したものと考えられる。また同時に白血球除去フィルターを用いての*T. cruzi*の通過性など検討した。その結果殆どのフィルターを*T. cruzi*虫体は通過してしまった。従って、残念ながら我が国で行われている現在の輸血用の保存血液提供システムでは、シャーガス病の輸血感染を確実には防止できない。ラテンアメリカ人に対する抗体チェックの実施とその強化などを、社会医学的影響を考慮の上、今後も更に安全輸血業務を遂行する為の対策の改善を図る必要がある。

| 報告企業の意見 | 今後の対応 |
|---|---|
| カーミC液(CPD液)を用いて*T. cruzi*感染マウス血液を4℃にて1～21日間保存処理を行ったところ、マウスへの感染性は無処置のものと差異は無かったが、病原性はかなり減弱していることが示唆された。我が国においても、安全輸血業務を遂行する為の対策の改善を図る必要があるとの報告である。 | 日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、国と協議しつつ対応を検討中である。今後も引き続き情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

二

12C－02

# 日本におけるラテンアメリカ人の慢性シャーガス病キャリアーからの献血についての対策検討

# The study of the counter measuers against blood donation from chronic Chagas disease carrier of latin-american residing in Japan

三浦　左千夫[1]、肥後　廣夫[2]、竹内　勤[1]
慶応義塾大学医学部熱帯医学寄生虫[1]、九州大学医学部感染免疫熱帯医学分野[2]

近年ラテンアメリカからの就労目的の定住化人口が増加の一途にあり、既に40万人を超えようとしている。当然就労目的の為、表向きは健常者としての来日である。しかし、就労中に疲れを訴え呼吸困難などの不調を来たした為に医療機関を受診し、出身地を考慮の後、血清免疫学的検査の結果シャーガス病感染を示唆された者が13名見いだされた。中には、末梢血で病原体*Trypanosoma cruzi*（*T.cruzi*）の存在を示唆する*T.cruzi*-DNAのPCR増幅断片が、検査のたびに検出される者もいた。また血液培養（LIT培地、NNN培地）で血液型虫体の分離にも成功した例など、慢性シャーガス病即ち病原体のキャリアーであることが明らかとなった者もいた。これらの抗体陽性者13名のうち１名については日本滞在中に献血を行っていた。注意すべきは、我が国では主要媒介昆虫が棲息しないものの、シャーガス病慢性キャリアーからの輸血、臓器移植などによる二次的感染の危険性である。今回、献血機関で保存血用に使用しているカーミC液（CPD液）を用いて感染マウス血液を４℃にて１～21日間保存処理をおこなった。これを正常マウスに接種し感染性、病原性ついて基礎的な検討を行った。その結果マウスへの感染性は無処置のものと差異は無かったが、病原性についてはかなり減弱していることが示唆された。これは*T.cruzi*に対して４℃という低温ストレスが影響したものと考えられる。また同時に白血球除去フィルターを用いての*T.cruzi*の通過性など検討した。その結果殆どのフィルターを*T.cruzi*虫体は通過してしまった。従って、残念ながら我が国で行われている現在の輸血用の保存血液提供システムでは、シャーガス病の輸血感染を確実には防止できない。ラテンアメリカ人に対する抗体チェックの実施とその強化などを、社会医学的影響を考慮の上、今後も更に安全輸血業務を遂行する為の対策の改善を図る必要がある。

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 10. 22 | 該当なし | |

| 一般的名称 | 人赤血球濃厚液 | 研究報告の公表状況 | | 公表国 |
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| 販売名(企業名) | 赤血球濃厚液-LR「日赤」(日本赤十字社)<br>照射赤血球濃厚液-LR「日赤」(日本赤十字社) | | Kleinman SH, Glynn SA, Lee TH, Tobler L, Montalvo L, Todd D, Kiss JE, Shyamala V, Busch MP; National Heart, Lung, Blood Institute Retrovirus Epidemiology Donor Study (REDS-II). Transfusion. 2007 Oct;47(10):1756-64. | 米国 |

**研究報告の概要**

○供血者のパルボ・ウイルスB19DNAの高感度PCRスクリーニング法による陽性率と定量測定

背景:供血者における高感度核酸検査法を用いたパルボ・ウイルスB19 DNAの陽性率は、血漿分画製剤製造部門で高力価ウイルスを検出するためにデザインされた検査の検出率よりも高いことが最近示されている。

試験デザインおよび方法:米国の血液センター7施設において2000年～2003年の期間に採取した5020名の供血血液から得られた保存血漿検体を検査した。50%検出限界(LOD)1.6 IU/mL(95%信頼区間[CI], 1.2～2.1 IU/mL)及び95% LOD 16.5 IU/ mL (95% CI, 10.6～33.9 IU/mL)のリアルタイムB19 DNA PCR法(PCR; TaqMan, Applied Biosystems)を用いて検査を実施した。B19 DNAの確認と測定は、別の2つの検体の再検査により行った。陽性が確定した検体は、FDAが承認した検査法を用いて抗B19免疫グロブリンM(IgM)及びIgGの有無をテストした。

結果:B19 DNA陽性率は0.88%(95% CI, 0.64%～1.2%)であった。B19 DNA力価が20 IU/mL以上であった供血者23名のDNA値は、中央値が105 IU/mL(四分位範囲42～481 IU/mL)であり、最高値が1869 IU/mLであった。B19 DNA陽性供血はいずれもIgG陽性であり、そのうちの10名(23%)はIgMも陽性であった。血清中のIgMが陽性であることは、DNA値の増加と関連付けられた($p = 0.0013$)。

結論:供血者のほぼ1%に低値のB19 DNAが検出された。IgM 及びIgG B19抗体のいずれも陽性であったDNA陽性供血(23%)は、急性感染症である可能性が高く、IgGが陽性であるがIgMが陰性の供血は、持続性のB19感染である可能性が高い。

**使用上の注意記載状況・その他参考事項等**

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 米国の供血者のほぼ1%に低値のヒトパルボウイルスB19 DNAが検出されたとの報告である。 | 今後も引き続き、ヒトパルボウイルスB19に関する新たな知見及び情報の収集に努める。日本赤十字社では、以前よりRHA法によるB19抗原検査を導入、ウイルス量の多い血液を排除している。今後は検査方法の改善によりさらなる感度向上を目指すこととしている。 |

(12)

# Prevalence and quantitation of parvovirus B19 DNA levels in blood donors with a sensitive polymerase chain reaction screening assay

*Steven H. Kleinman, Simone A. Glynn, Tzong-Hae Lee, Leslie Tobler, Leilani Montalvo, Deborah Todd, Joseph E. Kiss, Venkatakrishna Shyamala, and Michael P. Busch for the National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study (REDS-II)*

**BACKGROUND:** Blood donor parvovirus B19 DNA prevalence with sensitive nucleic acid test assays has recently been demonstrated to be higher than that found with assays designed to detect high viral titers in the plasma manufacturing sector.

**STUDY DESIGN AND METHODS:** Stored plasma aliquots from 5020 donations collected between 2000 and 2003 at seven US blood centers were tested. Testing was performed with a real-time B19 DNA polymerase chain reaction (PCR; TaqMan, Applied Biosystems) assay with a 50 percent limit of detection (LOD) of 1.6 IU per mL (95% confidence interval [CI], 1.2-2.1 IU/mL) and a 95 percent LOD of 16.5 IU per mL (95% CI, 10.6-33.9 IU/mL). Confirmation and quantitation of B19 DNA was accomplished by retesting of two additional subaliquots. Confirmed-positive specimens were tested for the presence of anti-B19 immunoglobulin M (IgM) and IgG with FDA-licensed assays.

**RESULTS:** B19 DNA prevalence was 0.88 percent (95% CI, 0.64%-1.2%). Among the 23 donations with B19 DNA titers of at least 20 IU per mL, the median DNA concentration was 105 IU per mL with an interquartile range of 42 to 481 IU per mL; the highest value was 1869 IU per mL. All B19 DNA–positive donations were positive for the presence of IgG and 10 (23%) were also positive for the presence of IgM; IgM seropositivity was associated with increasing DNA levels ($p = 0.0013$).

**CONCLUSION:** Low-level B19 DNA was detected in nearly 1 percent of donations. The 23 percent of DNA-positive donations with both IgM and IgG B19 antibody most likely represent acute resolving infection, whereas those with IgG but no IgM are most consistent with a more chronic and possibly persistent phase of B19 infection.

Parvovirus B19 infection (also known as human erythrovirus and referred to as B19 in this report) has been well documented to be transmitted by transfusion of plasma derivatives.[1-3] There are only rare case reports, however, of B19 transmission by transfusion of blood components, and two small studies that attempted to assess such transmission systematically did not demonstrate any symptomatic infection.[4-9] To date, there have been no large-scale linked transfusion transmission studies with sufficient statistical power to allow for a systematic calculation of the per unit or per recipient risk of acquiring asymptomatic or symptomatic infection after transfusion of a B19-viremic blood component.

In the plasma derivative setting, B19 transfusion transmission has not been reported when the plasma B19 DNA concentration was less than $10^3$ international units

**ABBREVIATIONS:** $C_T$ = cycle threshold; LOD = limit of detection.

From Westat, Rockville, Maryland; Blood Systems Research Institute, San Francisco, California; the Institute for Transfusion Medicine, Pittsburgh, Pennsylvania; Chiron Corp., Emeryville, California; Digene Corp., Gaithersburg, Maryland; and the National Heart, Lung, and Blood Institute, Bethesda, Maryland.

*Address reprint requests to:* George Schreiber, ScD, Westat, 1650 Research Boulevard, Rockville, MD 20850; e-mail: GeorgeSchreiber@westat.com.

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(IU) per mL.[1] It is unknown if recipients of pooled plasma products with low B19 viral titers are protected due to the neutralizing effect of B19 antibody from other units in the plasma pool, the low B19 viral titer, or a combination of both.[10] Although it has been assumed that single-unit blood components with low B19 DNA titers should, similarly, be noninfectious, this remains speculative because the mechanism of protection in the pooled plasma setting has not been established and may not apply to single-unit transfusions.

Newer information suggests that the potential for recipients to be exposed to low titers of B19 DNA from blood component transfusion is greater than previously thought. Through the use of sensitive nucleic acid test (NAT) assays, two sets of investigators have found that the prevalence of B19 DNA in donor plasma ranges from 0.5 to 0.9 percent.[11,12] Furthermore, it is now known that B19 DNA may persist in plasma at low concentration for several years in healthy individuals who could make repeat blood donations during this viremic interval.[13-16]

From 2000 through 2003, NHLBI and CDC established the Retrovirus Epidemiology Donor Study Allogeneic Donor and Recipient (RADAR) repository as a powerful tool to investigate possible transfusion-transmitted infections.[17] Our primary aim with regard to B19 infection was to use this linked donor and recipient repository to evaluate whether donations with low B19 DNA levels transmit infection. Such a transfusion transmission study would only be feasible, however, if a B19 NAT assay with appropriate performance characteristics (e.g., sensitivity, specificity, throughput) was available and if the prevalence of plasma B19 DNA in the donor population and the number of susceptible recipients were of sufficient magnitude to allow for significant conclusions to be drawn.

The primary aim of this report is to present the results of these initial investigations, which include development of a highly sensitive polymerase chain reaction (PCR) screening assay and estimation of the prevalence of plasma B19 DNA in donations represented in the RADAR repository. Our secondary aims were to evaluate the association of quantitative DNA levels with immunoglobulin M (IgM) and IgG antibody status and determine the demographic characteristics of B19 DNA–positive donors.

## MATERIALS AND METHODS

### Selection of repository specimens

The RADAR repository was established from 2000 through 2003 through participation of blood centers and selected hospitals at seven geographically dispersed locations throughout the United States.[17] This donor and recipient repository contains pretransfusion (or peritransfusion) specimens and follow-up specimens (collected at an interval of 6-12 months) from 3,575 enrolled recipients. The repository also contains 13,201 donation specimens given by 12,408 distinct donors that were transfused to these RADAR recipients. This portion of the repository is referred to as the linked donor-recipient repository. In addition, there is a supplementary repository of 99,906 donation specimens (contributed by 84,339 donors) from donations that were not transfused to enrolled RADAR recipients.

As previously reported, transfusion transmission studies with the RADAR-linked repository should usually only be considered if the donor prevalence of an agent is at least 0.05 percent.[17] For this study, based on some of the conservative estimates of donor B19 viremia in the literature, we determined that testing of approximately 5,000 specimens would allow us to be 95 percent confident that the prevalence of viremia in the donor population was at least 0.05 percent. Thus, 5,200 specimens (allowing for failed runs) were selected from the repository of unlinked community whole-blood and apheresis donations for B19 DNA PCR testing. A stratified sampling procedure was used to select these specimens so that they would have similar demographic, temporal, and geographic characteristics to the 13,198 community whole-blood and apheresis donation samples in the linked repository, thereby allowing for later extrapolation of the prevalence results to donations in the linked repository. The sampling scheme controlled for frequency of donations per donor, blood center where donation was given, and year and month of donation, in that order. This stratification also ensured that the distributions of other important variables, for example, age at time of donation, first-time or repeat status, and race/ethnicity were similar between the 5,200 sampled unlinked donations and the 13,198 donation samples in the linked repository. The similarity of the sampled supplementary repository subset and the linked donations was verified after the sample was selected.

A 1.75-mL frozen plasma tube for each selected donation was accessed from the repository by personnel at the long-term storage facility (SeraCare BioServices, Gaithersburg, MD). Each specimen was aliquoted into three 0.5-mL subaliquots (one for B19 DNA screening and two for B19 DNA confirmation and quantitation) and one 0.25-mL aliquot (for antibody testing) with rigorous precautions to minimize the possibility of cross-sample contamination.

### PCR assay development, validation, and performance characteristics

The B19 DNA assay used in this study was originally developed by Chiron Corp. (Emeryville, CA) and subsequently refined through a collaboration between Chiron and Blood Systems Research Institute (San Francisco, CA).[18] The assay format includes a magnetic-bead B19 DNA capture step followed by a real-time PCR assay that targets the VP1 region of the B19 genome. An internal control, sharing homologous primer region sequences but with a different internal probe binding sequence as the viral

target, is included in each assay tube. B19 DNA target and the internal control nucleic acid are amplified by the same primer pair but detected and distinguished by fluorophore-tagged sequence-specific probes. Five-hundred microliters of frozen plasma, thawed at room temperature, was vortexed and centrifuged briefly before the addition of lysis buffer, poly(T)-coupled magnetic beads (Seradyn, Indianapolis, IN), four viral capture primers (VSCP1, VSCP4, VSCP5, and VSCP7) with poly(A) tail, and 20 copies of internal control. The preparation was vortexed for 10 seconds and incubated in a 60°C water bath for 20 minutes, followed by incubation at room temperature for 15 minutes. The tubes were placed on a magnetic base for 10 minutes before the liquid was vacuum-aspirated. The beads were washed once with 1 mL of wash buffer (Procleix, Gen-Probe, San Diego, CA) and twice with another wash buffer (Chiron Novartis, Emeryville, CA).

All captured target DNA from the 0.5-mL input plasma and captured spiked internal control were subjected to amplification in a single PCR procedure and amplification and detection occurred in a 96-well optical plate with dual-plexed TaqMan PCR technology. TaqMan 1000 Rxn PCR core reagents were purchased from Applied Biosystems (Foster City, CA). The PCR mix was prepared by mixing 10 µL of Buffer A; 1 µL of the enzyme uracil-*N*-glycosylase (Amperase [Roche Diagnostics, Indianapolis, IN], which reduces contamination by degrading dUTP-containing amplicons from prior amplification reactions); 20 µL of $MgCl_2$; 10 µL of dATP, dCTP, dGTP, and dUTP; 0.5 µL of AmpliTaq Gold; 56 µL of sterilized water; 0.9 µL each of two amplification primers at 100 pmol per µL (VSCP8, VSCP9); and 0.25 µL of each of the two probes at 100 pmol per µL (VSCP10, VB-TAM) per sample. One-hundred microliters of the mix was added to each sample instead of the manufacturer-suggested 50 µL per sample. PCR was performed with 50 cycles of 95°C for 15 seconds and 60°C for 1 minute, after the initial Amperase (50°C for 2 min) and AmpliTaq Gold activation (95°C for 10 min). The DNA was amplified and detected with a real-time PCR system (ABI 7500, Applied Biosystems).

Features of the assay system that minimize risk of specimen-to-specimen cross-contamination of plasma or "carryover" amplicon contamination include single-tube magnetic bead target-capture and DNA purification with the Chiron/Gen-Probe–enhanced semiautomated system, single-tube amplification, and real-time monitoring of fluorescent probe binding to amplicon products with no subsequent manipulation of reaction wells; use of dUTP and UNG in each assay to destroy previous B19 amplicons before amplification; and single-use disposable reaction tubes and plates. Segregated laboratories were used for sample accessioning and preparation, preamplification target-capture, and real-time PCR.

Preliminary assay development work used a series of dilutions of the CBER parvovirus B19 DNA standard to determine where to set the assay cutoff as well as to estimate the resultant assay analytic sensitivity. Figure 1 shows box and whisker plots of testing results for 30 replicates at each of four dilutions (30, 15, 7.5, and 3.75 IU/mL). Based on these studies, the assay cutoff was established as follows: a specimen was classified as reactive if a signal was detected at not more than 40 cycles (cycle threshold [$C_T$] ≤ 40), indeterminate if $C_T$ was more than 40 but not more than 45, and negative if there was no signal detected or if a $C_T$ was more than 45. An apparent negative result was interpreted as invalid if the $C_T$ of the internal control was more than 45.

Because the chosen assay cutoff was designed to maximize assay sensitivity, an algorithm was developed for final test interpretation so as to avoid classifying nonspecific reactivity on a single assay run as a confirmed-positive result (see Fig. 2). All initially positive, initially indeterminate, and invalid specimens were retested in duplicate with two separate 0.5-mL subaliquots on plates that included quantitative run standards. This testing

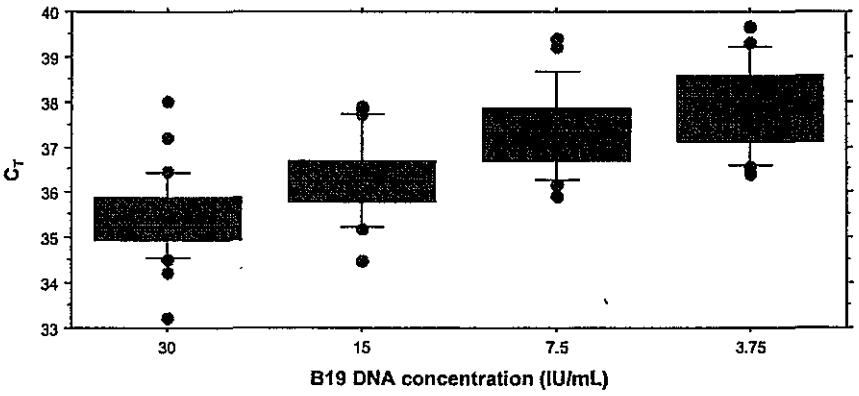


**Fig. 1. Assay validation and selection of assay cutoff based on replicate testing of dilutions of the CBER parvovirus B19 DNA standard. The input per amplification was 0.5 mL. Twofold dilutions of CBER-validated B19 DNA standard were run with 30 replicates at each concentration. The standard was diluted with pooled plasma negative for B19 DNA and B19 antibody. The y-axis represents PCR $C_T$. The x-axis represents B19 DNA concentration per milliliter of plasma. The top and bottom whiskers on the box plot represent the 90th and 10th percentiles, respectively. The top of the box represents the 75th percentile and the bottom of the box represents the 25th percentile. The line inside the box represents the median. Negative plasma aliquots were also tested (data not shown). Of 204 B19-negative plasma aliquots, 203 yielded negative assay results ($C_T$ > 40). One negative control sample amplified at 36.30 $C_T$.**

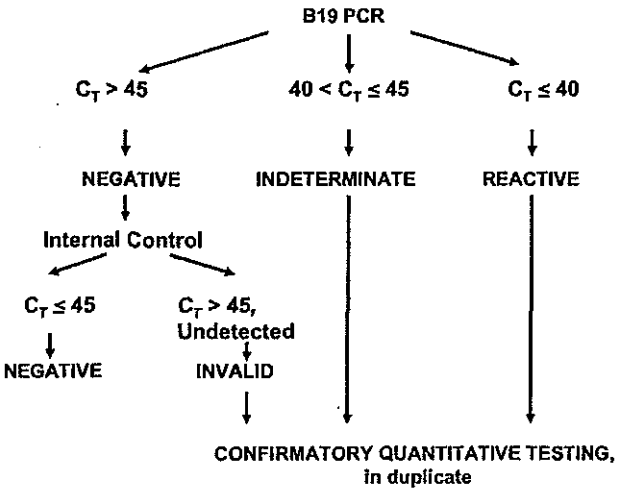


**Fig. 2. B19 DNA testing algorithm.**

served both as confirmation and as quantitation. The final interpretation of the qualitative PCR assay was based on the results of the three individual assays (i.e., the initial screening assay and the duplicate repeat assays). Specimens were classified as B19 DNA–positive if at least two of three tests showed reactivity at a $C_T$ of not more than 40 cycles and indeterminate if at least two tests showed reactivity at a $C_T$ of not more than 45, with one or both of these $C_T$ values more than 40, and negative if both of the duplicate repeat assays were negative.

For determining DNA concentration, quantitative run standards were placed on each plate in duplicate. For confirmation and quantitation of initially reactive specimens, standards from 1000 to 31.25 IU per mL were tested in twofold dilutions. For repeat testing of indeterminate and invalid specimens, standards from 125 to 31.25 IU per mL were tested in twofold dilutions to prevent any cross-contamination of the specimens from high-titer standards. Quantitative results were determined by comparing the $C_T$ of the specimen to the $C_T$ of the known standards on the same test run. The assigned quantitative value for each specimen was the average of the two duplicate quantitative assays (including zero for a negative test result). Specimens with low $C_T$ ($C_T$ < 30) were diluted 1:10 and 1:100 and then run in triplicate at each dilution, and the quantitative result was the mean of the three test results at the most appropriate dilution.

Analysis of additional replicates of the CBER standard (30-60 replicates performed on twofold serial dilutions with concentrations of 30, 15, 7.5, 3.75, 1.88, and 0.94 IU/mL) established that the 50 percent limit of detection (LOD) of the assay was 1.6 IU per mL (95% confidence interval [CI], 1.2-2.1 IU/mL), and the 95 percent LOD was 16.5 IU per mL (95% CI, 10.6-33.9 IU/mL). To allow for the possibility that quantitation might not be precise at the lower limits of detection, we categorized all specimens with quantitative values of 0 to less than 20 IU per mL as having a value of less than 20 IU per mL.

### PCR testing of study specimens

Initial B19 DNA testing was performed in singlicate with one 0.5-mL plasma aliquot. Testing was performed in 96-well microtiter plates. Each plate contained two known positive, two blinded negative, and two blinded positive controls as well as up to 90 study specimens. All positive controls were prepared by the testing laboratory (BSRI) from the CBER parvovirus B19 DNA standard and were diluted to contain 100 IU per mL B19 DNA. The known controls were introduced into each test batch by the testing laboratory whereas the blinded controls were introduced into each specimen batch by the repository facility. Runs were considered valid if at least one of the two known positive and one of the two known negative controls gave a valid, expected result. Figure 3 shows the high consistency of assay performance on the known and blinded positive control specimens for 56 screening test runs based on $C_T$; the $C_T$ for the known controls (Fig. 3A) was 33.36 ± 2.96 and the $C_T$ for the blinded controls at the same concentration was 34.09 ± 2.71 (Fig. 3B). All positive controls reacted with the exception of 5 of 112 known positive controls with invalid results and 1 of 112 blinded positive controls with a false-negative result. In addition, 110 of 112 negative controls were negative, 1 gave an invalid result, and 1 gave an indeterminate result.

All initially positive, indeterminate, and invalid specimens were rerun in duplicate with two separate subaliquots on plates that included quantitative run standards. Because of limitations of specimen volume, this testing served both as confirmation and as quantitation (see above).

### B19 antibody testing

All confirmed B19 DNA–positive and indeterminate donations were tested for the presence of B19 IgG and IgM antibodies against a recombinant VP2 protein with FDA-licensed test kits (Biotrin, Dublin, Ireland). Testing was performed in singlicate with the 0.25-mL subaliquot. If results fell into the equivocal zone, the assay was repeated in singlicate and the repeat result was taken as the overall final result for the specimen.

Additionally, to determine IgG and IgM prevalence in B19 DNA–negative donations, we first randomly selected a subset of 520 donation specimens from the 5200 donations that had been selected for PCR testing (see above). This sampling occurred before obtaining the PCR results on the 5200 donations. IgG antibody testing was performed on 505 of the 520 donations, 501 of which were subsequently found to be B19 DNA–negative. For IgM antibody, due to kit availability issues, a random subset of

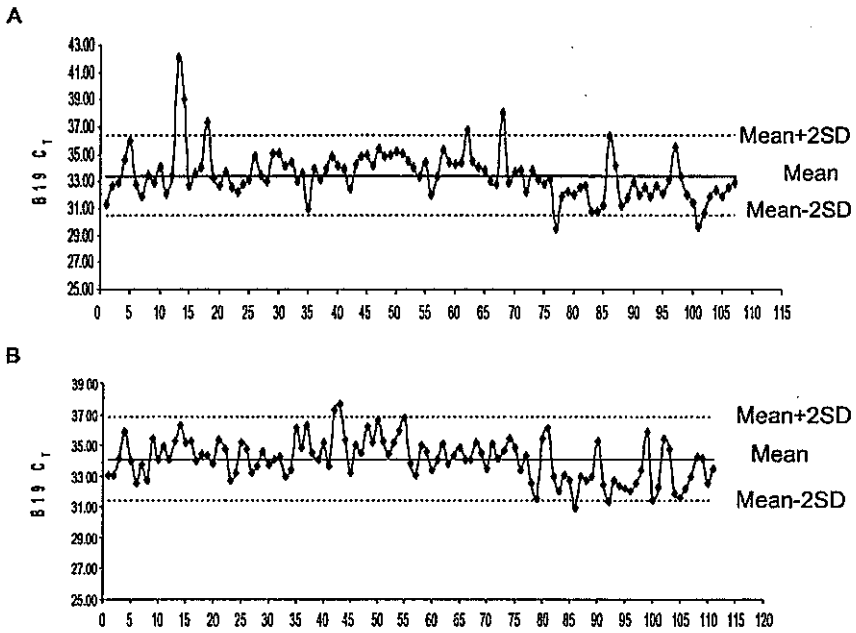


**Fig. 3. (A) Control chart: 100 IU per mL known controls. Two known positive controls were included per run. The data plotted were controls for 56 plates, which includes 107 valid results and excludes 5 invalid results ($C_T$ mean ± 2SD = 33.36 ± 2.96). (B) Control chart: 100 IU per mL blinded controls. Two blinded positive controls were included per run along with two blinded negative controls (data not shown). The data plotted were positive controls for 56 plates, which includes 111 valid results and one false-negative result ($C_T$ mean ± 2SD = 34.09 ± 2.71).**

194 of 366 of these specimens identified as IgG-positive were tested.

### Demographics

The following information was available for each donation in the RADAR repository: donor identification number, age at time of donation (categorized as ≤25, 26-35, 36-45, 46-55, 56-65, ≥66), sex, race/ethnicity (Asian, black non-Hispanic, Hispanic, white non-Hispanic, other non-Hispanic), first-time or repeat donor status, education level (<high school, high school degree, some college education, college degree, graduate or professional degree), history of transfusion, the center at which the donation was collected, and date of donation (categorized by calendar year of donation or by season, i.e., occurring in the winter, spring, summer, and fall).

### Statistical analysis

We calculated the proportion of donations that were confirmed positive by PCR with associated 95 percent CI as well as the prevalence of IgM and IgG (and their 95% CI) in DNA-positive and DNA-negative donations. We evaluated whether the prevalence of IgM in DNA-positive donations varied as a function of B19 DNA level (categorized as <20, 20 to $<10^2$, $10^2$ to $<10^3$, and $10^3$ to $<10^4$ IU/mL) by conducting a Fisher's exact test (SAS/STAT 9.1, 2004, SAS Institute, Inc.). We determined the 50 and 95 percent LOD of our PCR assay with associated 95 percent CI by probit analysis with PC SAS Version 8.2.

We compared the distribution of demographic characteristics between donation groups (i.e., DNA-positive and DNA-negative donations) with chi-square statistics or, for small cell sizes, either the Fisher's exact test (SAS/STAT 9.1, SAS Institute, Inc.) or the Fisher-Freeman-Halton test (StatXact Version 6, 2004, Cytel Software Corp., Cambridge, MA). This latter test is a generalization of the Fisher's exact test for 2 by 2, to an r-by-c contingency table.

## RESULTS

Of the 5200 specimens originally selected for DNA testing, screening results were obtained for 5020. Results were not obtained for 180 specimens that were part of two runs which failed due to equipment problems. There were 113 initially reactive specimens (2.25%), 26 initially indeterminate specimens (0.52%), 56 initially invalid specimens (1.12%), and 4825 negative specimens. After retesting, 43 of the initially reactive specimens confirmed as positive, 2 were reclassified as indeterminate, and 68 were reclassified as negative. For the indeterminate specimens, 1 was reclassified as positive, 22 remained indeterminate, and 3 became negative. All initially invalid specimens retested as negative.

Summarizing the screening and retesting results, we found that 44 specimens (0.88%) were DNA-positive, 5 (0.10%) were indeterminate, and 4971 (99.02%) were negative. In 35 (80%) of the confirmed-positive specimens, all three tested replicates reacted in the PCR assay. DNA prevalence was 0.88 percent with a 95 percent CI of 0.64 to 1.2 percent.

Figure 4 shows that the percentage of confirmed-positive specimens was inversely related to the $C_T$ value obtained on the initial screening test run. Specimens that initially reacted at a $C_T$ value of less than 37 were confirmed as positive 86 percent of the time, whereas specimens with a $C_T$ value of between 37 and 40 were confirmed 16 percent of the time.

Table 1 presents the quantitative DNA levels grouped into four categories as well as the antibody status of the 44 B19 DNA confirmed-positive donations. The median DNA level for all of our confirmed-positive donors was 22.75 IU

per mL. Twenty-one of 44 specimens had DNA levels of less than 20 IU per mL (conservatively determined to be the lower limit of quantitation of the assay), and if we consider only those donors who had DNA levels of more than 20 IU per mL, then the median DNA level for these 23 donors was 105 IU per mL (interquartile range, 42-481 IU/mL), with the highest value being 1869 IU per mL. Specimens with reactivity on two of the three replicates had lower DNA levels than specimens reactive on all three replicates (data not shown).

All B19 DNA confirmed-positive donations had detectable B19 IgG antibody, whereas in the control group of 501 PCR-negative donors, IgG was present in 73 percent (95% CI, 68%-77%). IgM antibody was detected in 10 B19 DNA confirmed-positive donations and was assigned an equivocal status in 2 additional cases. IgM seropositivity was associated with increasing DNA concentration ($p = 0.0013$). The median DNA level for the 10 IgM-positive donations was 297 IU per mL, and all three donors with B19 DNA titers of more than $10^3$ IU per mL were IgM-positive. IgM was not detected in any of 194 DNA-negative, IgG-positive donors (95% CI, 0.00%-1.88%).

Donors who were not more than 45 years old were more likely to be viremic than donors older than 45 years:

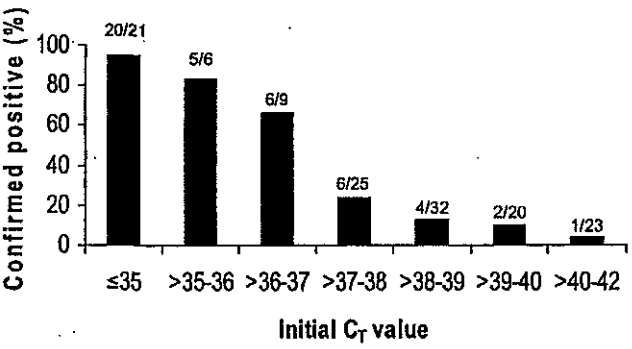


**Fig. 4. Confirmation of B19 DNA reactivity relative to the $C_T$ obtained on initial PCR screening. Confirmation of all 136 initially reactive specimens. There were no initially reactive specimens with $C_T$ between 42 and 45 cycles. All 4884 specimens with a $C_T$ value of more than 45 on the initial test run were classified as negative.**

1.24, 1.30, and 1.41 percent of donors not more than 25, 26 to 35, and 36 to 45 years, respectively, were viremic compared to 0.23, 0.37, and 0.00 percent of donors 46 to 55, 56 to 65, and more than 65 years old ($p = 0.0008$). Further, 1.85 percent of first-time donors were viremic compared to 0.66 percent of repeat donors ($p = 0.007$). Although DNA prevalence estimates appeared to be higher for spring (1.14%) and summer (1.18%) donations than for fall (0.44%) and winter (0.67%) donations, these differences did not achieve significance ($p = 0.09$). There was no significant association with geographic region (based on center where the donation was given), calendar year of donation, sex, race/ethnicity, education level, or transfusion history.

## DISCUSSION

In this study we applied a highly sensitive B19 DNA assay to 5020 individual donations to determine the prevalence of plasma B19 DNA in donors from seven different geographic areas of the United States using specimens collected over a 4-year interval. We believe this to be the largest such study performed on individual donations rather than on large pools of donations, as is routine in the plasma manufacturing sector.

We found that the prevalence of B19 DNA in plasma was 0.88 percent with a 95 percent CI of 0.64 to 1.2 percent. Our data can be compared with several recent European studies that used somewhat less sensitive B19 DNA assays. Thomas and coworkers[11] tested 16,859 Belgian blood donors in pools of 60 donations with an assay with 95 percent LOD of 96.6 IU per mL and found a B19 DNA prevalence of 0.16 percent. Candotti and colleagues[12] tested 1,000 UK whole-blood and platelet donors in minipools of 10 donation specimens and found a prevalence of 0.9 percent with a nested PCR with a 95 percent LOD of 25 IU per mL; this prevalence was very similar to that found in our study. Plentz and colleagues[8] found a 0.7 percent prevalence with an assay with a 50 percent LOD of 60 to 80 copies per mL when retrospectively testing 1,806 blood products transfused in a hematology ward. In contrast, studies reported in the plasma manufacturing sector have reported much lower prevalence, ranging from 0.008 to 0.04 percent; it is notable that these

**TABLE 1. Quantitative PCR and antibody results on confirmed-positive specimens***

| Viral load (IU/mL) | Number of confirmed-positive specimens | IgM-positive and IgG-positive | IgM-equivocal and IgG-positive | IgG-positive only |
|---|---|---|---|---|
| <20 | 21 | 1 (5) | 1 (5) | 19 (90) |
| 20 to $<10^2$ | 11 | 2 (18) | 0 (0) | 9 (82) |
| $10^2$ to $<10^3$ | 9 | 4 (44) | 1 (11) | 4 (44) |
| $10^3$ to $<10^4$ | 3 | 3 (100) | 0 (0) | 0 (0) |
| Total | 44 | 10 (23) | 2 (4) | 32 (73) |

* Data are reported as number (%).

studies used NAT assays that were designed to lack sensitivity so as to only detect units from donors in the stage of acute viremia with DNA concentrations of more than $10^5$ or $10^6$ IU per mL.[19-21]

The generally accepted understanding of the natural history of B19 infection in immunocompetent individuals such as blood donors states that viremia occurs approximately 1 week after infection and persists in high titer for approximately 5 days. With the development of IgM antibody at approximately 12 days after infection (followed within days by IgG antibody), viremia levels drop precipitously and viremia usually disappears within weeks.[22,23] IgM antibody becomes undetectable after several months (although this precise duration is unknown) but IgG persists long term and is thought to convey immunity to reinfection. As a variation of this usual natural history, plasma viremia may persist for more than 6 months to several years in some cases, and recent data suggest that B19 may persist in other tissue sites (e.g., skin, synovia) for a much longer period of time in a significant percentage of individuals.[13-16,24]

The antibody findings in our study can be used to assess the stage of viremia that we detected in our B19 DNA–positive donors. We did not detect any B19 DNA–positive donors who lacked both IgM and IgG antibody nor did we detect any donors with a DNA concentration at or above $10^5$ or $10^6$ IU per mL, which would be characteristic of the several-day interval after infection. Given the low rate of detection of high-titer DNA in previous studies, it is not surprising that with the testing of 5020 donations in this study, we did not detect any such donations. We also did not detect any donors in the short window period where IgM antibody is present but IgG is absent. We detected 10 donors who were positive for the presence of IgM and IgG (23% of the 44 B19 DNA–positive donors) and an additional 2 who were IgM-equivocal. These donors were probably in a relatively early stage of infection, that is, within the first several months of acquiring infection. Consistent with the known natural history of B19 infection, the IgM-positive, DNA-positive donors had higher DNA levels than the IgM-negative, DNA-positive donors. We detected 32 DNA-positive donors who were IgG-positive only: 28 of these had DNA levels of less than $10^2$ IU per mL; the median DNA level in these donors (as for all 44 DNA-positive donors) was lower than that previously reported by other investigators. We believe either that these DNA-positive, IgG-positive donors were at the tail end of resolving their B19 viremia or that some of these donors may have had very-low-titer B19 DNA that persisted for longer than predicted by the standard natural history model. Future longitudinal studies will be needed to distinguish these possibilities. The 73 percent prevalence of B19 IgG seropositivity and the lack of IgM antibody in our B19 DNA–negative control donors were consistent with reports in other donor cohorts.[11,12,25]

B19 infections are known to occur with a spring and summer preponderance and to vary in annual frequency in cycles that span several years.[25] In our study, the B19 DNA prevalence was higher in spring and summer donations, but did not achieve significance. This may be due to limitations in sample size or alternatively to our use of a highly sensitive NAT assay that may have allowed us to detect B19 DNA–positive donors for a relatively long period of time after acquisition of infection, thereby masking the expected temporal findings. The significant association with younger age (<age 46) may correlate with an increased likelihood of possible B19 exposure in young adults from contact with young children or with a lower degree of susceptibility among previously infected older adults. First-time donors showed higher prevalence of viremia than repeat donors, which may in part reflect the younger age distribution of first-time donors or may be due to other unexplained factors as has been seen with other infectious agents (e.g., human immunodeficiency virus, hepatitis C virus) in the donor population.[26]

The real-time B19 TaqMan PCR assay used in this study gave reproducible results on known standards, had a low failed run rate, gave a low rate of invalid specimens due to internal control failure, and showed no evidence of sample cross-contamination. Furthermore, the assay had a high analytic sensitivity at the chosen cutoff (50% LOD, 1.6 IU/mL; 95% LOD, 16.5 IU/mL). Although our choice of a relatively high $C_T$ cutoff introduced nonspecificity on initial testing (presumably due to nonspecific probe binding), our confirmatory algorithm minimized false-positive results by requiring a reactive result on a second aliquot subjected to the full extraction, amplification, and detection procedure before designating the donation as confirmed positive for the presence of B19 DNA.

At present, interventions for preventing B19 transfusion from blood components have not been implemented in the vast majority of developed countries, due in part to the prevailing view that blood components with low levels of B19 DNA will not transmit B19 infection. Recently several authors have cited the need for studies to verify this hypothesis.[11,12] The results of the study reported in this article have established that there is sufficient statistical power to carry out such a B19 transfusion transmission study with the real-time B19 TaqMan PCR assay to test specimens in the RADAR repository. This conclusion is based on the demonstrated donor B19 DNA prevalence of 0.88 percent combined with a sufficient number of B19-susceptible recipients in the RADAR repository; that is, we observed that 22 percent of tested RADAR recipients were B19 IgG–negative on their pretransfusion specimen (data not shown), which was similar to the 27 percent prevalence of IgG seronegativity in tested donors. We are now actively engaged in per-

forming a linked B19 transfusion transmission study using the RADAR repository.


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# 医薬品　研究報告 調査報告書

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**研究報告の概要**

ヒトパルボウイルス B19（B19V）は血液試料に多く認められるヒト病原体であり、主に呼吸器経路を介して伝播される。B19V は他のパルボウイルスよりも物理化学的な処理に対する感受性が高いが、その理由は未だ明らかではない。
血漿プールは数百の血液で構成されるため、PCR 法で検査すると大半の血漿プールから B19V DNA が検出され、第 VIII および第 IX 凝固因子、ヒト血清アルブミン、静注用免疫グロブリン、筋注用免疫グロブリン、プロトロンビン複合体濃縮製剤、アンチトロンビン III などで報告があるが、いずれも B19V DNA を検出したことを証明しているのであって、必ずしも感染性のあるウイルスの存在を証明しているわけではない。しかし、血漿分画製剤による伝播の報告もある。
パルボウイルスは最も安定なウイルス群に属し、物理化学的な処理の多くに抵抗性であるが、B19V は乾熱または湿熱のほか、低 pH または高 pH、UVC 照射、光化学反応により不活化できる。
本報告では B19V 不活化能について「60℃・10 分」および「pH4・2 時間」の 2 条件で評価したところ、ウイルスの感染性は低下し検出限界未満となり、既発表データと一致していた。
熱または低 pH による B19V 不活化機序は、DNA を包むカプシドの分解ではなく、カプシドからの DNA の遊離によるものであることがわかったが、熱安定化剤としてクエン酸を用いると B19V DNA はカプシドから遊離せず、感染性が維持される。

| 報告企業の意見 | 今後の対応 |
|---|---|
| ヒトパルボウイルス B19 の不活化について「60℃・10 分」および「pH4・2 時間」の 2 条件で評価したところ、感染性は検出限界未満となり、既発表データと一致していたとの報告で、他のパルボウイルスに比べ不活化されやすいとも述べられている。<br>なお、弊社血漿分画製剤は最終製品において核酸増幅検査によりヒトパルボウイルス B19 DNA が陰性であることを確認している。 | 今後ともヒトパルボウイルス B19 に関する血漿分画製剤の安全性に関する情報に留意していく。 |

**使用上の注意記載状況・その他参考事項等**

**慎重投与の項**
・溶血性・失血性貧血の患者［ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。］
・免疫不全患者・免疫抑制状態の患者［ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。］

**重要な基本的注意の項**
(1) 本剤の原材料となる‥［スクリーニング項目、不活化・除去工程］‥投与に際しては、次の点に十分注意すること。
1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。

**妊婦、産婦、授乳婦等への投与の項**
妊婦又は妊娠している可能性のある婦人には治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。［妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。］

# Molecular mechanism underlying B19 virus inactivation and comparison to other parvoviruses

*Bernhard Mani, Marco Gerber, Patricia Lieby, Nicola Boschetti, Christoph Kempf, and Carlos Ros*

**BACKGROUND:** B19 virus (B19V) is a human pathogen frequently present in blood specimens. Transmission of the virus occurs mainly via the respiratory route, but it has also been shown to occur through the administration of contaminated plasma-derived products. *Parvoviridae* are highly resistant to physicochemical treatments; however, B19V is more vulnerable than the rest of parvoviruses. The molecular mechanism governing the inactivation of B19V and the reason for its higher vulnerability remain unknown.

**STUDY DESIGN AND METHODS:** After inactivation of B19V by wet heat and low pH, the integrity of the viral capsid was examined by immunoprecipitation with two monoclonal antibodies directed to the N-terminal of VP1 and to a conformational epitope in VP2. The accessibility of the viral DNA was quantitatively analyzed by a hybridization-extension assay and by nuclease treatment.

**RESULTS:** The integrity of the viral particles was maintained during the inactivation procedure; however, the capsids became totally depleted of viral DNA. The DNA-depleted capsids, although not infectious, were able to attach to target cells. Comparison studies with other members of the *Parvoviridae* family revealed a remarkable instability of B19V DNA in its encapsidated state.

**CONCLUSION:** Inactivation of B19V by heat or low pH is not mediated by capsid disintegration but by the conversion of the infectious virions into DNA-depleted capsids. The high instability of the viral DNA in its encapsidated state is an exclusive feature of B19V, which explains its lower resistance to inactivation treatments.

B19 virus (B19V) is the only well documented human pathogen of the *Parvoviridae* family. The virus belongs to the genus *Erythrovirus*. In most cases, the infection is either asymptomatic or accompanied by mild nonspecific symptoms. The most common syndrome caused by B19V is an erythematous rash illness named erythema infectiosum affecting children. B19V is also the causative agent for transient aplastic crisis, which may have severe effects on patients suffering from sickle cell disease and other anemic illnesses. Chronic infections accompanied by pure red cell aplasia and anemia affect immunocompromised patients. Furthermore, B19V may cause fetal death, autoimmune diseases, and arthropathies.[1]

B19V is a widespread pathogen. The serologic evidence of a past infection is 40 to 60 percent for young adults and 80 to 100 percent for elder people.[1,2] Owing to its high prevalence, blood donations are frequently contaminated with B19V. The measured incidence of contamination depends on the sensitivity of the detection method and ranges from 0.003 percent (immunodiffusion) to 1.2 percent (polymerase chain reaction [PCR]) of blood donations examined.[3-7] Because plasma pools are constituted of hundreds of donations, B19V DNA is found in the majority of plasma pools as determined by PCR.[8-10] The contamination of plasma-derived products, such as coagulation factors VIII and IX, human serum albumin, intravenous immune globulin, intramuscularly injected

ABBREVIATIONS: B19V = B19 virus; MVM = minute virus of mice; PBSA = phosphate-buffered saline containing 1 percent bovine serum albumin; $PLA_2$ = phospholipase A2.

From the Department of Chemistry and Biochemistry, University of Bern; and CSL Behring AG, Bern, Switzerland.

*Address reprint requests to:* Carlos Ros, Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland; e-mail: carlos.ros@ibc.unibe.ch.

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immune globulin, prothrombin complex concentrate and antithrombin III has been reported.[8,9,11,12] Therefore, there is a risk of transmitting B19V through the administration of plasma-derived products. In these studies however, the contamination was demonstrated with the presence of B19V DNA with PCR, which does not necessarily prove the presence of infectious virus. Nevertheless, direct evidence of B19V transmission through the administration of plasma-derived products has also been shown in several case studies.[13-16] Moreover, patients that receive such medication on a regular basis show a higher prevalence of B19V-specific antibodies than control groups.[17] Altogether, the contamination of plasma-derived products indicates a potential risk of a B19V infection for the treated patient with potentially severe consequences for pregnant women and anemic and immunocompromised patients.

To achieve maximal safety for plasma-derived clinical products, pathogen safety guidelines have been established, as a result of which manufacturers must demonstrate the effective elimination of viral agents during the manufacturing process of their products. Virus elimination is demonstrated either with the relevant pathogen itself or with one or several closely related model viruses. To date, there is no convenient cell culture infectivity test for B19V. For this reason, animal parvoviruses such as bovine parvovirus, canine parvovirus, porcine parvovirus, or minute virus of mice (MVM) are often used for validation studies regarding the inactivation of B19V. Parvoviruses are among the most stable viruses and have been shown to resist many common physicochemical inactivation procedures. B19V inactivation can be achieved with dry or wet heat,[18-21] as well as with low or high pH,[22,23] UVC irradiation,[24,25] or photochemical reactions.[26] Interestingly, B19V has been found to be more readily inactivated than other parvoviruses. Whereas B19V is inactivated beyond the detection limit after 10 minutes at 60°C or after 2 hours at pH 4, canine parvovirus,[21] MVM,[27] and porcine parvovirus[18] can withstand 1 hour at 60°C without considerable inactivation. Similarly, the treatment of MVM at pH 4 for 6 hours only moderately reduces its infectivity.[23] The reason why B19V is more sensitive to inactivation than other parvoviruses is not known. Although different inactivation conditions for B19V have been described, the underlying mechanism of B19V inactivation has not yet been elucidated. It is generally assumed that the inactivation occurs through capsid disintegration because the viral genome becomes accessible to DNases.[18,27] We have shown in a recent study, however, that after mild heat treatments, the DNA from B19V and MVM can be rendered accessible without capsid disintegration.[28]

In this study we have analyzed the B19V capsid rearrangements occurring during the inactivation process. The results revealed a sequence of structural transitions preceding capsid disintegration. The critical transition, which resulted in full virus inactivation, was the dissociation of the viral DNA from the still intact capsid. Comparison studies revealed that the DNA release from intact capsids is a common feature among parvoviruses but occurs much more prematurely in B19V, explaining its lower resistance to inactivation procedures.

## MATERIALS AND METHODS

### Cells and viruses

Human UT7/EPO cells were propagated in RPMI 1640 supplemented with 5 percent fetal calf serum (FCS) and 2 U per mL recombinant human erythropoietin (EPO; Janssen-Cilag, Midrand, South Africa) at 37°C and 5 percent $CO_2$. UT7 cells were provided by A. Gröner (CSL Behring, Marburg, Germany). Two B19V-containing plasma samples (Genotype 1) were obtained from two infected individuals (S-1 and S-2) and did not contain B19V-specific immunoglobulin M or immunoglobulin G (IgG) antibodies. B19V was concentrated from infected serum by ultracentrifugation through 20 percent sucrose. The viral pellet was washed and resuspended in phosphate-buffered saline (PBS). All other parvoviruses were derived from cell culture supernatant. H-1 parvovirus was provided by C. Dinsart (German Cancer Research Center, Heidelberg, Germany). Porcine parvovirus was provided by T. Novak (CSL Behring, Marburg, Germany).

### Exposure of viral particles to inactivation conditions

Viral suspensions in PBS were heat-treated in thin-wall tubes for 3 or 10 minutes in a preheated thermoblock. A probe was used to monitor the temperature of the suspension. After the temperature treatment, the samples were rapidly cooled on ice and immediately used for subsequent reactions. For pH treatments, the viral suspensions were acidified by adding MES-buffered saline until the desired pH was achieved and incubated for 2 hours at 37°C. After the treatment, the pH of the viral suspension was neutralized by dilution (1:100) into PBS or in PBS containing 1 percent BSA (PBSA). Additionally, the heat sensitivity of B19V in citrate buffer, which has been recently reported to confer heat resistance to B19V,[28] was examined. The viral suspension was diluted in citrate buffer (0.5 mol/L trisodium citrate, 0.1 mol/L NaCl, pH 7) or in PBS and exposed to heat as specified above.

### Infectivity assay

Titration of B19V was performed by limited dilution in quadruplicate. UT7 cells were seeded on 96-well plates ($3 \times 10^4$ per well) in RPMI, containing 2 U per mL recombinant human EPO and 5 percent FCS. Virus was diluted geometrically by the factor 10 in RPMI. An equal volume of

diluted virus was added to each well and incubated at 37°C in 5 percent $CO_2$. After 4 days, the cell culture volume was carefully removed and cells were fixed with a solution of ice-cold methanol:acetone (1:1, v/v) for 1 hour at 20°C. After fixation, the cells were air-dried, washed with PBSA, and incubated with a mouse antibody against B19V (1:40 diluted in PBSA, clone R92F6 $IgG_1$, Novocastra, Newcastle upon Tyne, UK) for 1 hour at room temperature. The cells were washed with PBSA, and as secondary antibody, a conjugated $F(ab')_2$ fragment of goat anti-mouse immunoglobulins was added (1:50 dilution, DakoCytomation, Glostrup, Denmark) for 1 hour at room temperature. After final washings with PBSA, the cells were overlaid with 50 µL of glycerin:PBS solution (1:1) and examined under fluorescence microscope. The infectivity titer was calculated with the Spaerman-Kärber method.[30]

### Assessment of B19V capsid integrity

After exposure to heat or low pH, the integrity of the viral capsid was examined by immunoprecipitation with two different antibodies. One antibody is directed to a VP2 conformational epitope (monoclonal antibody [MoAb] 860-55D), which exclusively recognizes capsids and not denatured proteins. Another antibody recognizes an epitope in the N-terminal of VP1 (MoAb 1418).[31] The immunoprecipitation was performed overnight at 4°C in the presence of 20 µL of protein G PLUS-agarose (Santa Cruz Biotechnology, Santa Cruz, CA) and 0.5 to 1 µg antibody in a total volume of 120 µL PBSA. The supernatant was carefully removed, and the beads were washed three times with PBSA. Immunoprecipitated viral capsids were resolved by sodium dodecyl sulfate (SDS)-10 percent polyacrylamide gel electrophoresis (PAGE). After the transfer to a polyvinylidene fluoride membrane, the blot was probed with a mouse anti-B19 VPs (1:500, US Biologicals, Swampscott, MA), followed by a horseradish peroxidase–conjugated secondary antibody (1:20,000 dilution). The viral structural proteins were visualized with a chemiluminescence system (Pierce, Rockford, IL).

### Assessment of B19V DNA accessibility

Subsequent to the temperature or pH treatments, the presence of externalized viral DNA was examined by a hybridization-extension assay as previously described.[28] Briefly, a probe consisting of a virus-specific 3'-end and a virus-unrelated 5'-end was hybridized to the target viral DNA and subsequently extended with sequenase (3.25 U, USB, Cleveland, OH). The extended probe was purified with a PCR purification kit (QIAquick, Qiagen, Valencia, CA) and quantified by real-time PCR. Alternatively, the presence of externalized viral DNA was examined by the treatment of the viral suspensions with DNase I (10 U, Amersham Biosciences, Piscataway, NJ) overnight at room temperature in PBS containing 6 mmol per L $MgCl_2$. The viral DNA was purified and quantified as specified below.

### Quantitative PCR

The viral DNA was quantified with a real-time PCR system (LightCycler, Roche Diagnostics, Rotkreuz, Switzerland). PCR was carried out with the FastStart DNA SYBR Green kit (Roche Diagnostics) following the manufacturer's instructions. For the detection and quantification of probe-extended DNA generated from the hybridization-extension reaction, a forward primer specific for the 5' virus-unrelated tail of the probe and a downstream virus-specific reverse primer were used. All probes and primers used are shown in Tables 1 through 3.

### Assessment of the viral DNA-capsid association

To verify whether the exposed viral DNA is still associated to the capsid or otherwise dissociated, the B19V capsids were immunoprecipitated with MoAb 860-55D as indicated above. The amount of viral capsid protein and viral DNA present in the immunoprecipitated and supernatant fractions was analyzed by SDS-PAGE and quantitative PCR, respectively.

### FACS analysis

The presence of B19V on the cell surface was quantitatively analyzed by flow cytometry. UT7/EPO cells were infected with either intact or heat-inactivated B19V (100 copies/cell) under conditions allowing the binding but not the internalization of the virus (4°C). The cells were washed three times and incubated with an anti-B19V capsid MoAb (5 µg/mL, 8293, Chemicon International, Temecula, CA) at 4°C for 1 hour in PBS containing 2 percent FCS, followed by an incubation with fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse IgG (5 µg/mL, A85-1, BD Biosciences, San Jose, CA) at 4°C for 1 hour. The cells were analyzed by flow cytometry with a flow cytometer (FACScan, Becton Dickinson, San Jose,

**TABLE 1. Probes used for the hybridization-extension assay**

| Virus | 5' virus-unrelated sequence | 3' virus-specific sequence |
|---|---|---|
| B19V | CGATCCGACTCACACCTGGACC..... | .....CCGCCTTATGCAAATG |
| BPV | GGGCGAAGAACGGTGGATTAA..... | .....CGAGGACAGGTGGACC |
| CPV | GGGCGAAGAACGGTGGATTAA..... | .....GCGGTTTGTGTGTTTA |
| H-1 | CCACAGAGGTCCAAGCACGCA..... | .....AGCGGTTCAGAGAGTT |
| MVM | GGGGATGCGGGGAGTGTACGGGC..... | .....GATAAGCGGTTCAGGG |
| PPV | AGGCGGTTCATGGGTGGATAG..... | .....GTTGCTTACTTCAGTT |

TABLE 2. Primers used for PCR after the hybridization-extension assay

| Virus | Forward primer | Reverse primer |
|---|---|---|
| B19V | CGATCCGACTCACACCTGGACC | CCCCGGTAAGGTCAAGCTTAGAAGC |
| BPV | GGGCGAAGAACGGTGGATTAA | CCCCCACATAGTTCATAGAAGCCT |
| CPV | GGGCGAAGAACGGTGGATTAA | TCCATTGCTGTTTGTGCTCCTGTA |
| H-1 | CCACAGAGGTCCAAGCACGCA | CCGCCCCTCGTTGTAGAGACTTC |
| MVM | GGGGATGCGGGGAGTGTACGGGC | CCAACCATCTGATCCAGTAAACAT |
| PPV | AGGCGGTTCATGGGTGGATAG | CCGTTTTGTGAGGCTCTCGATT |

TABLE 3. Primers used for B19V genome detection

| Forward primer | Reverse primer |
|---|---|
| TGGGGCAGCATGTGTTAAA | CACAGGTACTCCAGGCACAG |

TABLE 4. Effect of temperature and low-pH treatments on B19V infectivity

| | S-1* | S-2 |
|---|---|---|
| Stock | 4.75† | 6 |
| pH 7.4‡ | 4.85 | 5.35 |
| pH 4 | ≤2.48 ≥2.37§ | ≤2.48 ≥2.87 |
| 37°C | 4.1 | 5.1 |
| 60°C | ≤2.48 ≥1.62 | ≤2.48 ≥2.62 |

* S-1 and S-2 are serum samples of two infected individuals.
† Titers are given in log $TCID_{50}$ per mL.
‡ pH and temperature treatments for 2 hours and 10 minutes, respectively.
§ Reduction of infectivity.

CA). Data acquisition and analysis were conducted with software (CellQuest Pro, BD Biosciences). The percentage of cells having B19V on their surface is indicated in the upper right quadrant of each panel.

## RESULTS

### B19V inactivation by heat and low-pH treatments

Two different conditions, 60°C for 10 minutes and pH 4 for 2 hours, were evaluated for their capacity to inactivate B19V. After these treatments, an immunofluorescence infectivity assay was performed as described above. The applied heat or low-pH treatments resulted in the reduction of the virus infectivity beyond the detection limit (Table 4). These results are consistent with previous data on the inactivation of B19V.[16,21,23]

### B19V inactivation by heat or low pH is not caused by capsid disintegration

Subsequent to the inactivation treatments by heat and low pH, the integrity of the viral capsid was examined. Viruses were immunoprecipitated with MoAb 860-55D against a VP2 conformational epitope, which recognizes only capsids.[31] The results showed that the inactivating heat treatments did not cause capsid disassembly (Fig. 1A). The capsid integrity was also examined with an antibody specific to N-VP1. As shown in Fig. 1A, after heat inactivation of B19V, VP2 could be immunoprecipitated with the antibody directed to N-VP1. Capsid disintegration was only observed increasing the incubation times at 60°C (Fig. 1B) or increasing the temperature above 60°C (Fig. 1C). As expected, treatments at 85°C resulted in the complete destruction of the viral capsids.

Similarly to the temperature treatment, inactivation of B19V by low-pH treatment was not caused by capsid disintegration. As shown in Fig. 1D, viral capsids remained assembled after exposure for 2 hours at pH 4. Moreover, exposure to more severe acidic conditions (pH 3) did not cause capsid disintegration.

### B19V inactivation by heat or low pH is due to the release of the viral DNA

After the heat and low-pH inactivation treatments, the accessibility of the viral DNA was examined with a hybridization-extension assay, as described above. The results showed that while the viral capsid remained assembled, the viral genome, however, became fully accessible. The amount of accessible viral DNA was similar to that detected after complete disintegration of the viral capsids at 85°C (Figs. 2A, 2B).

To determine whether the DNA that had become accessible by the inactivation treatments was still associated with the virus capsid or otherwise dissociated, viruses were immunoprecipitated with the MoAb against capsids, and the DNA content in the supernatant and immunoprecipitated fractions was determined with quantitative PCR. As expected, in the untreated virus samples, all the viral DNA was immunoprecipitated and only a minor amount of DNA was detectable in the supernatant. Exposure of viruses to the temperature of 60°C or higher, however, resulted in total release of the viral DNA from the capsids (Fig. 2C). The same results were obtained after inactivation at pH 4 for 2 hours (Fig. 2D), indicating that the inactivation mechanism of B19V by heat or low-pH treatments was similarly caused by the conversion of the infectious DNA-containing virions into noninfectious empty capsids.

### B19V DNA is not externalized and the infectivity is preserved when using citrate as thermostabilizer

It has been recently reported that in the presence of citrate, B19V becomes resistant to inactivation by pasteurization. Citrate is used as a protein stabilizer in the preparation of some plasma-derived products.[29] The mechanism by which the presence of citrate considerably

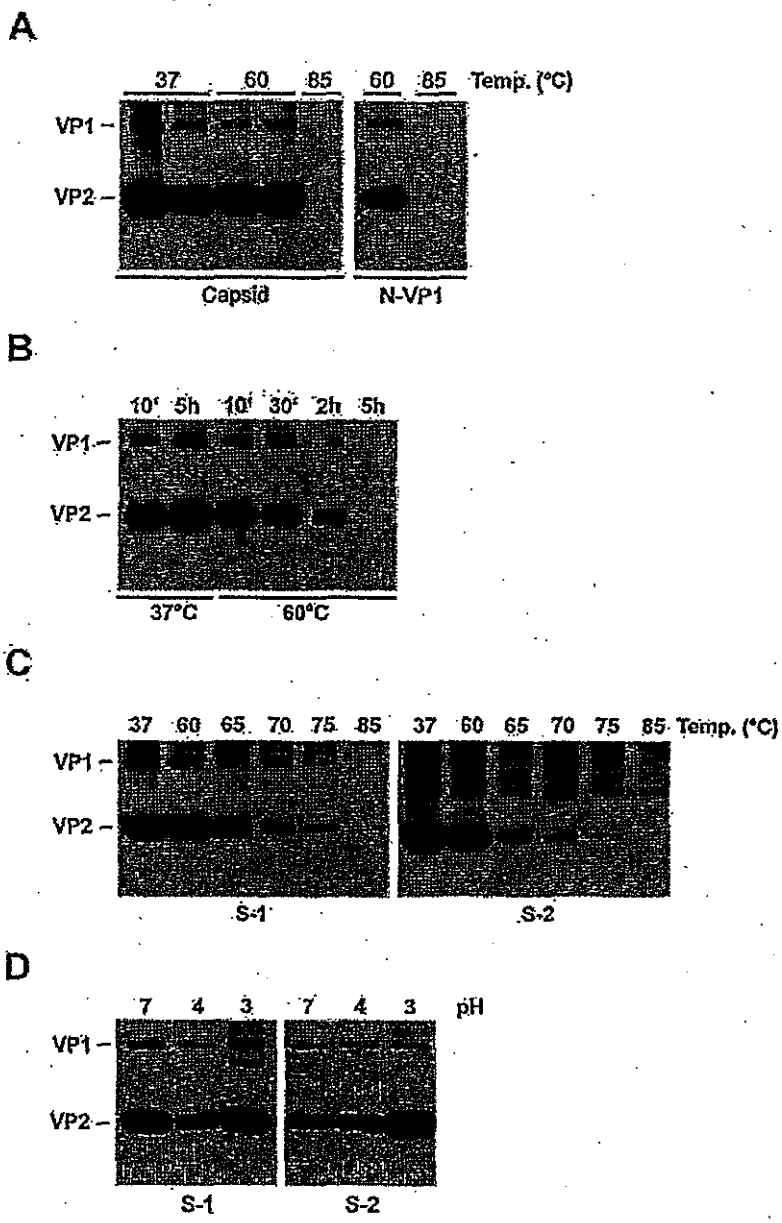


**Fig. 1. Effect of inactivation by heat or low pH on B19V capsid integrity. After the exposure of B19V to different conditions, the intact capsids were immunoprecipitated and analyzed by Western blot. The immunoprecipitation was performed with an antibody directed to a VP2 conformational epitope (MoAb 860-55D), except for the right section in A, where an antibody recognizing an epitope in the N-terminal of VP1 (MoAb 1418) was used.[31] The immunoprecipitations were performed after exposure to (A) 60°C for 10 minutes, (B) increasing incubation times at 60°C, (C) increasing incubation temperatures, and (D) after exposure to low pH.**

increases the heat resistance of B19V remains unknown. We have examined and compared the heat sensitivity of B19V in PBS and in a buffer containing citrate, as specified under Materials and Methods. The results confirmed that although the virus was fully inactivated in PBS, the presence of citrate conferred heat resistance and the virus could not be inactivated (data not shown). As expected, the viral DNA became fully accessible after the heat treatment of B19V in PBS but was not externalized in the presence of citrate (Fig. 3).

### The inactivated DNA-depleted capsids preserve their capacity to bind cells

The capacity of the heat-inactivated B19V particles to bind the target cells was tested. The same amount of inactivated and infectious B19V was added to UT7 cells under conditions that allowed only viral binding and not internalization (4°C). Subsequently, flow cytometry analysis was performed with a B19V capsid proteins antibody as described above. The results revealed that the heat-inactivated and the infectious B19V bound to UT7 cells with a similar efficiency (Fig. 4).

### B19V shows a unique DNA externalization pattern among parvoviruses

B19V is more readily inactivated than other parvoviruses. To understand the reason for this difference, the externalization of the B19V DNA was compared to that of other parvoviruses. B19V, bovine parvovirus, canine parvovirus, H1, MVM, and porcine parvovirus were exposed to increasing temperatures for 3 minutes, and the amount of accessible DNA was determined with the hybridization-extension assay. The rate of externalization was remarkably similar among all the examined viruses except for B19V (Fig. 5). At 50°C, approximately 40 percent of the B19V virions externalized their DNA, whereas barely any externalized DNA could be detected in the case of the other par-

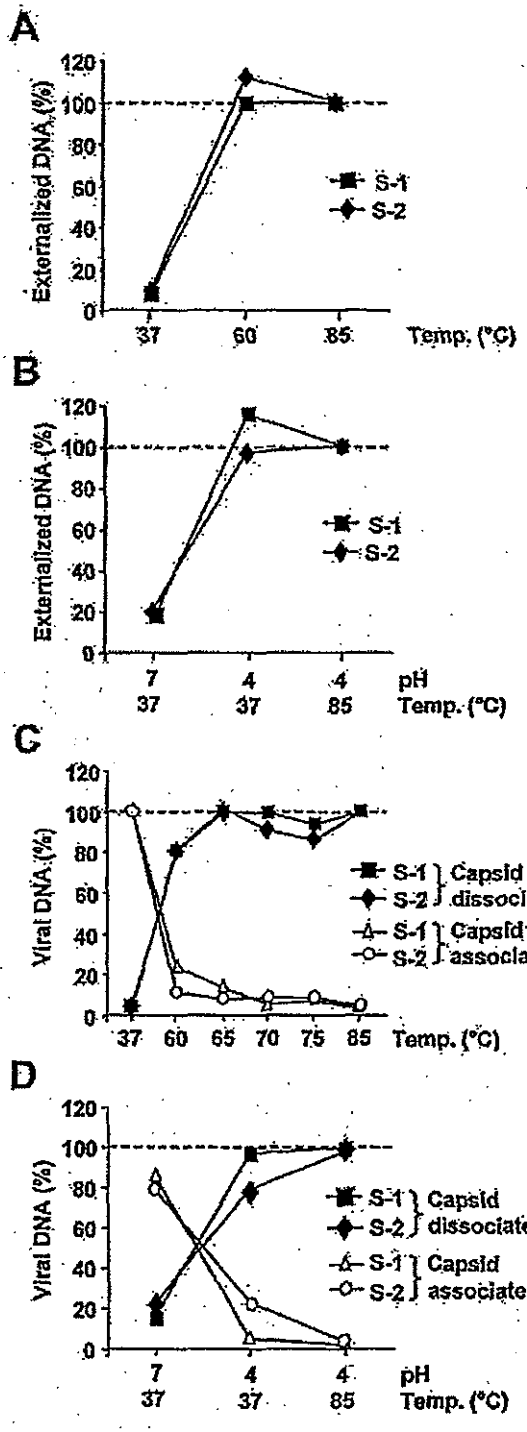


Fig. 2. Effect of inactivation by heat or low pH on B19V DNA accessibility and release. (A, B) Effect of inactivation on B19V DNA accessibility. The externalized DNA (%) refers to the amount detected at 85°C. (C, D) Effect of inactivation on B19V DNA release (dissociation from the capsid). Viral DNA (%) in relation to the input is shown.

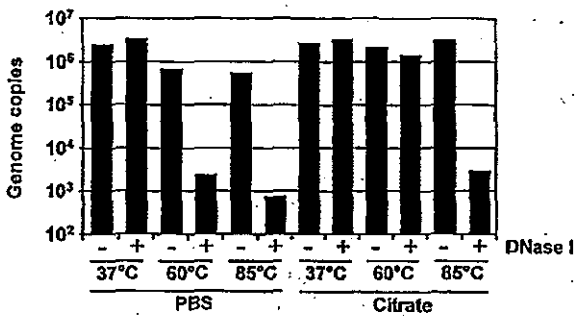


Fig. 3. Sensitivity of B19V DNA to DNase I after heat treatment in PBS or in citrate buffer.

voviruses. Although 60°C treatment leads to the externalization of nearly all the B19V genomes, the externalization in the rest of the tested viruses was at approximately 20 percent and in the range of 40 to 80 percent at 70°C. These results imply that the reason for the faster inactivation of B19V is due to the higher instability of its DNA in the encapsidated state.

## DISCUSSION

To date, the lack of an appropriate cell culture to propagate B19V has complicated the experimental work with this virus. In contrast, optimal cell systems are available for many animal parvoviruses. For this reason, they are commonly used in validation studies as models for B19V. For an unknown reason, however, B19V has been shown to be more easily inactivated than the other members of the *Parvoviridae* family.[22,23] Therefore, the animal parvoviruses do not mimic the effect of inactivation procedures on B19V.[32] Although different inactivation conditions for B19V have been described, the underlying mechanism of the inactivation and the reason for its higher vulnerability to physicochemical conditions have not yet been elucidated.

In this study we have examined the structural capsid rearrangements occurring during the inactivation of B19V. For this purpose, we have applied two different procedures previously shown to efficiently inactivate B19V.[18,21,27] One is the exposure of the virus to heat (60°C for 10 min) and the other is the exposure to acidic conditions (pH 4 for 2 hr). Our results demonstrated that the first structural transition determining B19V inactivation is not the disintegration of the capsid, which remained intact (Fig. 1), but the loss of the viral DNA (Fig. 2). Interestingly, the heat sensitivity of B19V largely depends on the composition of the buffer. In a recent report, it was shown that a solution containing citrate conferred heat resistance to B19V.[29]

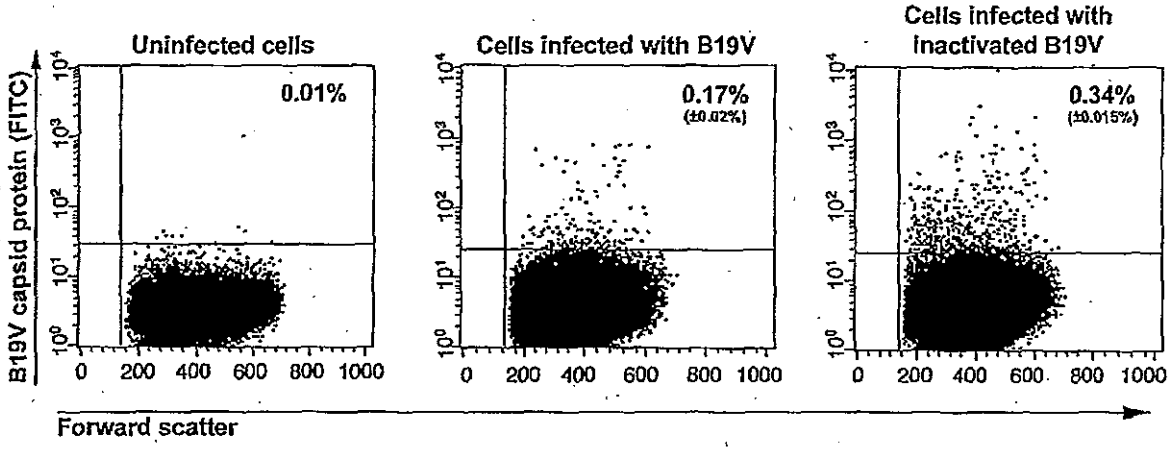


**Fig. 4. Capacity of inactivated virus to bind to susceptible cells. UT7 cells were infected with either untreated or heat-inactivated B19V. The proportion of cells with bound virus was determined with FACS and is shown in the upper right quadrant of each panel. The percentages represent the mean ± SD of three separate experiments.**

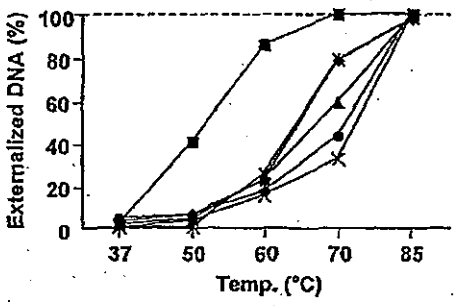


**Fig. 5. DNA externalization pattern of different parvoviruses in response to increasing temperatures for 3 minutes. The amount of externalized DNA was quantified with the hybridization-extension assay. Values of DNA (%) refer to the total amount detected after 85°C treatment, which was in the range of $10^5$ to $10^6$ molecules per microliter for all viruses. (■) B19V; (●) bovine parvovirus; (◆) canine parvovirus; (▲) H1 parvovirus; (×) MVM; (✳) porcine parvovirus.**

In our studies, we have confirmed this observation and found that in the presence of citrate, the viral DNA remains encapsidated (Fig. 3).

The release of the viral DNA in response to heat treatment was also detected in other parvoviruses (Fig. 5). Quantitative studies revealed that the kinetics of DNA externalization were surprisingly similar in all tested viruses with the exception of B19V, where it occurred prematurely (Fig. 5). The remarkable instability of the viral DNA in its encapsidated conformation explains the lower resistance of B19V to inactivation treatments.

The mechanism by which the intracellular environment destabilizes the parvovirus particles resulting in the release of the viral DNA is not fully understood. Growing evidence, however, indicates that parvovirus uncoating is performed without the need to disassemble the highly rigid capsid.[26,33-35] A series of capsid transitions triggered by the low endosomal pH seems to play a critical role by rendering the capsid flexible enough to allow the release of the viral DNA.[35] Among these transitions is the exposure of N-VP1. Increasing experimental evidence suggests that the conformational change leading to N-VP1 externalization leads also to DNA externalization.[33,34] Sustaining this notion is the observation that under mild acidification (pH 5), B19V externalizes N-VP1 sequences, and the viral DNA becomes accessible although mostly associated with the capsid.[28,36] In contrast, low pH treatment of MVM externalizes neither the N-VP1[34] nor the viral DNA.[28] Figure 6 represents schematically the progressive capsid rearrangement steps occurring during the inactivation of B19V.

As a result of the inactivation conditions applied in the present study, two major viral components were generated, empty capsids and free viral DNA, which might still have certain biologic activity. It has been recently shown that free genomic Kilham rat virus DNA induces innate immune activation and autoimmune diabetes through the TLR9 pathway;[37] however, whether B19V DNA or capsid proteins stimulate the innate immune system is not known. It has been increasingly acknowledged that pathogenic manifestations of B19V can also be elicited by the virus capsid proteins alone without infection. For instance, it has been shown that VP2 proteins are able to block hematopoiesis in vitro and in vivo.[38] The phospholipase A2 ($PLA_2$) activity of B19V is thought to contribute to inflammatory and autoimmune manifestations[39,40] and is suspected to be responsible for the arthropathies caused by B19V as well.[41] Although internal in native capsids, the VP1-$PLA_2$ motif becomes accessible upon exposure to

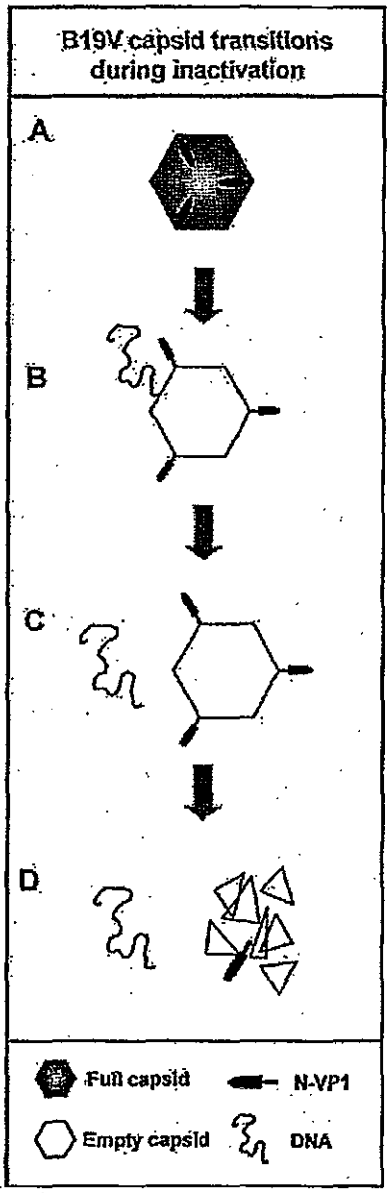


**Fig. 6. Schematic representation of the B19V capsid structural transitions during inactivation. The first structural rearrangements observed after mild heat or low-pH treatments of B19V is the externalization of N-VP1 sequences,[36] including the $PLA_2$ motif and the accessibility of the viral DNA.[28] At higher temperatures or more acidic conditions, the viral DNA is dissociated from the capsid. Finally, the viral particle is disintegrated.**

heat or low pH.[28] Therefore, although not infectious, the inactivated capsids are enzymatically active. The binding of the $PLA_2$-active capsids to cells (Fig. 4), whether specific or not, might still have certain biologic effect. It seems very unlikely, however, that such effects could be elicited through the administration of plasma-derived products containing inactivated B19V intact capsids. First, there may not be any intact capsids present in plasma-derived products due to the application of procedures of virus removal and/or inactivation, which are by far stronger than the ones applied in the present studies. Second, to elicit biologic activities other than virus replication, a large amount of B19V capsids or genomic viral DNA would be required. Synoviocyte migration for instance has been shown only to occur at a concentration of $10^{11}$ virions per mL.[41] Also, Norbeck and colleagues[38] use $10^{12}$ protein molecules per mL in an assay that showed the inhibition of hematopoiesis by VP2. Such high concentrations are simply not possible in plasma-derived products.

In summary, the molecular mechanism underlying the inactivation of B19V has been elucidated. The first structural transition determining B19V inactivation is not the disintegration of the capsid but the release of the viral DNA. Comparison studies revealed that although the DNA release from intact capsids seems to be a common feature within the *Parvoviridae* family, it occurs much more promptly and to a higher extent in B19V, explaining its lower resistance to inactivation treatments.

ACKNOWLEDGMENT

The authors acknowledge S. Modrow (Regensburg, Germany) for providing the monoclonal antibodies 860-55D and 1418 against capsids and N-VP1, respectively.

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**研究報告の概要**

<背景及び目的>パルボウイルスは小さな非エンベロープの DNA ウイルスで、ウイルス不活化処理に対して比較的抵抗性がある。最近確認されたヒトパルボウイルス PARV4 が、類縁のジェノタイプ 2 型ウイルス（PARV5）を含め、血漿分画製剤の製造に使用されたプール血漿に混入していることが分かった。本報告では PARV4 が凝固因子製剤中に存在するのか否かを決定するための調査について述べる。
<材料及び方法>過去 30～35 年間に製造された第Ⅷ因子製剤について PARV4 及び B19 シーケンスのスクリーニングを実施した。PARV4 陽性製剤中の PARV4 ウイルス量は TaqMAN 分析法で測定し、DNA シーケンス分析によりジェノタイプを確認した。
<結果>第Ⅷ因子製剤 175 ロットのうち 28 ロットが PARV4 シークエンスを含み、その内 2 ロットにジェノタイプ 1 型及び 2 型の両方が存在することが分かった。最大ウイルス量は 10^5copies/mL 以上であった。PARV4 陽性の第Ⅷ因子製剤の大部分は 1970 年代及び 1980 年代に製造されていた。B19 もまたこれらの製剤をしばしば汚染していた。
調査した 175 ロットの PARV4 DNA 陽性ロットを有効期間別に区分すると、有効期間が 1974-1989 年のロットでは 23%（27/115）が陽性であったのに対して、1990-2005 年では 2%（1/60）が陽性であった。
<結論>PARV4 は第Ⅷ因子製剤の 16%、特に 1970 年代及び 1980 年代の古いロットから検出された。これらの製剤からのウイルス安全性及びレシピエントへの感染可能性の重要性は、依然不明である。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 過去 30～35 年間に製造された第Ⅷ因子製剤から PARV4 シークエンスが検出されたとの報告である。<br>PARV4 が発見されたのは 2005 年であり、PARV4 及びその関連変異型である PARV5 の病原性は現時点では明らかではない。今後も注意深く PRV4 に関する追加情報をフォローする必要があると考える。 | PRV4 に関連する情報については、今後も注視することとする。 |

**使用上の注意記載状況・その他参考事項等**

1. 慎重投与（次の患者には慎重に投与すること）
(4) 溶血性・失血性貧血の患者〔ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には，発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(5) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には，持続性の貧血を起こすことがある。〕
2. 重要な基本的注意
(1) 略
1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルスＢ19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
5. 妊婦，産婦，授乳婦等への投与妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルスＢ19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産，胎児水腫，胎児死亡）が起こる可能性がある。〕

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ORIGINAL PAPER


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# Human parvovirus PARV4 in clotting factor VIII concentrates

J. F. Fryer, A. R. Hubbard & S. A. Baylis

*Division of Virology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK*

**Background and Objectives** Parvoviruses are small non-enveloped DNA viruses, relatively resistant to virus inactivation procedures. The recently identified human parvovirus PARV4, including a related genotype 2 virus (also termed PARV5), has been found to be a contaminant of pooled plasma used in the manufacture of plasma-derived products. This report describes an investigation to determine whether PARV4 is present in clotting factor concentrates.

**Materials and Methods** Factor VIII concentrates manufactured in the past 30–35 years were screened for PARV4 and human parvovirus B19 (B19V) sequences. Viral loads in products testing positive for PARV4 were quantified using a consensus TaqMan assay designed to a highly conserved region. DNA sequence analysis was performed to confirm the genotypes present.

**Results** From a total of 175 lots of factor VIII concentrate, 28 of these contained PARV4 sequences, and in two lots both genotypes 1 and 2 were found to be present. The highest viral loads observed exceeded $10^5$ copies per ml. The majority of factor VIII concentrates testing positive for PARV4 were manufactured in the 1970s and 1980s. Human B19V was also a frequent contaminant of these products.

**Conclusions** PARV4 was detected in 16% of factor VIII concentrates, particularly in older batches from the 1970s and 1980s. The significance in terms of the viral safety and potential transmission to recipients of these products is not yet known.

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**Key words:** factor VIII, genotype, PARV4, PARV5, parvovirus, virus contamination.

## Introduction

PARV4 was originally identified in plasma from a patient with symptoms of acute virus infection following high-risk behaviour for human immunodeficiency virus 1 (HIV-1) transmission, but subsequently confirmed to be HIV-1 negative [1]. This patient was an intravenous drug user, infected with hepatitis B virus (HBV), with a range of symptoms including fatigue, vomiting and diarrhoea, sore throat, neck stiffness and joint pains. Phylogenetic analysis showed that PARV4 did not closely resemble other known human or animal parvoviruses [1].

Parvovirus B19 (B19V) is the prototype human parvovirus, infecting erythroid progenitor cells leading to erythema infectiosum, aplastic crisis, arthropathy and hydrops fetalis [2]. B19V is normally transmitted via the respiratory route; however, transmission also occurs through the administration of contaminated blood products and solvent/detergent-treated plasma and can result in clinically apparent infection [3–6]. Since 2004, European regulations have required that manufacturers of certain plasma derivatives, including anti-D immunoglobulin and plasma pooled and treated for virus inactivation, screen pooled plasma for B19V by nucleic acid amplification techniques (NAT), and this has led to a reduction in the levels of B19V present in manufacturing start pools [7]. NAT screening for B19V has now been widely implemented by manufacturers.

We have recently demonstrated the presence of PARV4 and a related variant virus (termed PARV5), in pooled plasma used in the manufacture of plasma-derived medicinal products [8]. These viruses are frequently detected in 4–5% of these pools with viral loads of up to $10^6$ copies per ml of plasma.

*Correspondence:* S. A. Baylis, Division of Virology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK
E-mail: sbaylis@nibsc.ac.uk

In the case of blood donors, PARV4 and PARV5 have been found in approximately 2% of individuals and at a higher frequency in febrile patients [9]. Sequence analysis shows that PARV4 and PARV5 share ~92% nucleotide identity over a 4860-bp region [10], similar to the level observed between B19V genotypes 1–3 [11], to which PARV4 shares ~45% nucleotide identity. At the amino acid level, PARV4 and PARV5 sequences are more conserved, and this is especially the case for the second open reading frame (ORF2), encoding the viral capsid-like protein, such that PARV4 and PARV5 are likely to represent a single serotype [10]. This sequence analysis has led to the proposal that PARV4 and PARV5 should be referred to by a single virus name, PARV4, comprising genotypes 1 and 2 (previously PARV5). In this study, we have investigated the presence of PARV4 genotypes 1 and 2 in clotting factor VIII concentrates, manufactured over the past 30–35 years. We have also examined these products for the presence of B19V.

## Materials and methods

### Factor VIII concentrates

Coagulation factor VIII concentrate products received at the National Institute for Biological Standards and Control (NIBSC) were stored at 4 to −20 °C until analysis. A total of 175 lots of 12 factor VIII concentrate products, from 10 manufacturers (named A–J), were investigated. Products were manufactured over a 30- to 35-year period, with expiry dates ranging between 1974 and 2005. Factor VIII product details are further described in Table 1.

### Nucleic acid extraction

Factor VIII concentrates were reconstituted in sterile distilled water according to the manufacturer's instructions. Total nucleic acid was extracted from 1 ml of reconstituted concentrate using the MagNA Pure LC instrument (Roche Applied Science, Mannheim, Germany) and was eluted in 50 μl as previously described [7].

### Screening for PARV4 in factor VIII concentrates

Factor VIII concentrates were initially screened for the presence of PARV4 genotype 1 and 2 sequences using a gel-based polymerase chain reaction (PCR), using primers specific to ORF2 of PARV4 [9]. We have previously confirmed the specificity and sensitivity of these primers to be one to 10 copies of PARV4 sequences. The presence of PARV4 in factor VIII concentrates was confirmed by DNA sequence analysis of amplification products. Amplicons were purified using the QIAEX Gel Extraction kit (Qiagen, Hilden, Germany). Sequencing was performed using the BigDye Terminator v1·1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK), using the T7 promoter primer and the pUC/M13 reverse primer. Following removal of dye terminators, using the DyeEx 2·0 Spin Kit (Qiagen), sequencing reactions were run on an ABI 3130XL Genetic Analyser (Applied Biosystems).

### Quantification of PARV4 in factor VIII concentrates

Following the initial screening of factor VIII concentrates for PARV4, viral loads in samples testing positive for these

Table 1 Detection of PARV4 and B19V in factor VIII concentrates

| Product/ manufacturer | Expiry date | Number of lots tested | Purification process | Virus inactivation | Number of positive lots by PCR | |
|---|---|---|---|---|---|---|
| | | | | | PARV4 | B19V |
| 1/A | 1974–1978 | 37 | Precipitation | None | 3 | 23 |
| 2/B | 1976–1977 | 2 | Precipitation | None | 1 | 2 |
| 3/C | 1976–1978 | 5 | Precipitation | None | 3 | 5 |
| 4/D | 1977–1978 | 2 | Precipitation | None | 1 | 2 |
| 5/E | 1977–1980 | 55 | Precipitation | None | 14 | 9 |
| 6/C | 1985 | 1 | Precipitation | Dry heat (68 °C, 72 h) | 1 | 1 |
| 4/F | 1985 | 1 | Precipitation and adsorption | Wet heat (heptane) (60 °C, 20 h) | 1 | 1 |
| 7/E | 1985–1987 | 8 | Precipitation and adsorption | Dry heat (68 °C, 72 h) | 0 | 5 |
| 8/A | 1986 | 4 | Precipitation (plus further purification) | Steam treatment (60 °C, 10 h) | 3 | 4 |
| 9/EGH | 1997–2004 | 16 | Monoclonal antibody | Pasteurization (60 °C, 10 h) | 0 | 2 |
| 10/I | 1998–2002 | 13 | Monoclonal antibody | Solvent/detergent | 0 | 7 |
| 11/I | 1999–2003 | 13 | Precipitation | Dry heat (80 °C, 72 h) | 1 | 7 |
| 12/J | 2001–2005 | 18 | Affinity chromatography | Solvent/detergent, dry heat (80 °C, 72 h) | 0 | 2 |
| | | | | Total number of positive lots/number of lots tested | 28/175 | 70/175 |

viruses were determined using a real-time PCR assay designed to a highly conserved region of PARV4 as previously described [9,10]. The primers used in this assay are directed towards a region of ORF2 of PARV4 that is highly conserved between the two genotypes. A standard curve was generated from plasmid DNA containing the 103-bp ORF2 PCR product.

### Detection of B19V DNA in factor VIII concentrates

Coagulation factor concentrates were additionally tested for the levels of B19V DNA using an in-house PCR assay as previously described [7]. This assay detects B19V genotypes 1–3.

### DNA sequence analysis of a variable region of ORF1 of PARV4

Using a multiple sequence alignment of near full-length PARV4 genomes (GenBank accession no. DQ873386-91) [10], primers were designed to a variable region of the PARV4 genome. Primers PARV35F (5′ TTCCTACTGGATTTCTCTCCAACC 3′) and PARV596R (5′ GGTAAGGCAATAGCACCTTGAGG 3′) were used to amplify a 562-bp region of ORF1 of PARV4 (corresponding to nucleotides 317–878 of PARV4 genotype 1, GenBank accession no. AY622943, and nucleotides 151–712 of PARV4 genotype 2, GenBank accession no. DQ873390), from extracted factor VIII samples. Amplification reactions were performed using the proof-reading enzyme Phusion™ Hot Start DNA Polymerase (Finnzymes OY, Espoo, Finland) as described previously [8]. For thermal cycling, a T3 thermal cycler (Biometra, Göttingen, Germany) was used with the following cycling conditions: 98 °C for 30 seconds, followed by 45 cycles of 98 °C for 10 seconds, 59 °C for 30 seconds and 72 °C for 20 seconds. Amplicons were analysed by agarose gel electrophoresis and compared with known size markers. Amplification products were purified as before, and cloned into the pT7 Blue vector according to the manufacturer's instructions (Novagen, Darmstadt, Germany). Sequencing was performed as previously described and was analysed using the GCG software package, version 10·2 (University of Wisconsin, Madison, WI, USA). Sequences were aligned using Clustal W [12], and a neighbour-joining tree (nucleotide distance with Jukes–Cantor correction, pairwise gap deletion) with bootstrap resampling (100 replicates), was constructed using MEGA3 software [13].

## Results

### Contamination of factor VIII concentrates with human parvoviruses

A total of 175 lots of 12 factor VIII concentrate products, from 10 manufacturers, were examined for the presence of PARV4 and B19V DNA by PCR. The expiry dates on these lots ranged from 1974 to 2005. As shown in Table 1, 16% (28/175) of lots tested positive for PARV4, while 40% (70/175) of lots tested positive for B19V DNA. The majority of factor VIII products testing positive for PARV4 DNA had an expiry date of pre-1990 [23% (27/115) of lots expiring 1974–1989 tested positive for PARV4, while only 2% (1/60) of lots expiring 1990–2005 tested positive for PARV4 DNA] (Fig. 1). In contrast, there was no significant difference in the prevalence of B19V in factor VIII products expiring pre- and post-1990 [45% (52/115) of lots expiring 1974–1989 tested positive for B19V, while 30% (18/60) of lots expiring 1990–2005 tested positive for B19V DNA] (Fig. 1).

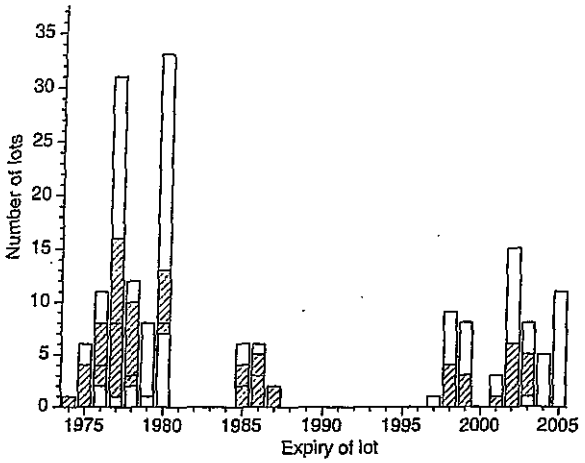


Fig. 1 Prevalence of parvoviruses PARV4 and B19V in factor VIII concentrates manufactured over the past 30–35 years. The number of lots testing positive for PARV4 (□), B19V (▨), both PARV4 and B19V (▨), and those testing negative for these viruses (□) are shown.

PARV4 ORF2 PCR products amplified by the gel-based assay were sequenced, and the majority determined to be of PARV4 genotype 2 (Table 2). In two factor VIII products both PARV4 genotype 1 and 2 sequences were amplified and sequenced. Viral loads of PARV4 in factor VIII products were determined by a consensus sequence real-time PCR assay [9], designed to detect a highly conserved region of ORF2 of PARV4. Viral loads ranged from < 100 to more than $3 \times 10^5$ copies per ml of product (Table 2), with the majority of contaminated lots containing 4–5 $\log_{10}$ PARV4 copies per ml of product (Fig. 2). The levels of B19V were as high as $2{\cdot}5 \times 10^8$ IU/ml of product (Table 2).

Manufacturing plasma pools relating to these factor VIII products were only available for the most recent factor VIII products. Factor VIII product number 28 (Table 2) had an expiry date of 2003, and was manufactured from two plasma pools 28A and 28B. Plasma pool 28A tested positive for PARV4 genotype 1 DNA by PCR with a viral load of $3{\cdot}3 \times 10^5$ copies per ml of plasma, while pool 28B tested negative for both PARV4 genotypes.

 

Table 2 Levels of PARV4 and B19V in factor VIII concentrates testing positive for PARV4 DNA

| Factor VIII | Product/ manufacturer | Expiry date | PARV4 genotype[a] | PARV4 viral load ($log_{10}$ genome copies per ml product) | B19V viral load ($log_{10}$ IU/ml product) |
|---|---|---|---|---|---|
| 1 | 1/A | 1976 | 2 | < 2·00[c] | 8·40 |
| 2 | | 1977 | 1 | 1·89 | 6·71 |
| 3 | | 1977 | 1 & 2[b] | 1·71 | 7·64 |
| 4 | 2/B | 1977 | 2 | 3·11 | 2·59 |
| 5 | 3/C | 1976 | 2 | 1·82 | 4·91 |
| 6 | | 1977 | 2 | 3·28 | 5·33 |
| 7 | | 1978 | 1 | 1·86 | 2·75 |
| 8 | 4/D | 1977 | 2 | 2·48 | 2·22 |
| 9 | 5/E | 1977 | 2 | 1·75 | – |
| 10 | | 1977 | 2 | 4·10 | 2·39 |
| 11 | | 1977 | 2 | 4·82 | 6·05 |
| 12 | | 1978 | 2 | 4·15 | – |
| 13 | | 1978 | 2 | 4·36 | – |
| 14 | | 1979 | 2 | 2·66 | – |
| 15 | | 1980 | 1 | 4·31 | 6·44 |
| 16 | | 1980 | 1 & 2 | 3·01 | – |
| 17 | | 1980 | 2 | 4·39 | – |
| 18 | | 1980 | 2 | 5·49 | – |
| 19 | | 1980 | 2 | 5·03 | – |
| 20 | | 1980 | 2 | 2·37 | – |
| 21 | | 1980 | 2 | 4·30 | – |
| 22 | | 1980 | 2 | 2·00 | – |
| 23 | 4/F | 1985 | 1 | < 2·00[c] | 4·57 |
| 24 | 6/C | 1985 | 1 | 1·32 | 5·79 |
| 25 | 8/A | 1986 | 1 | 4·08 | 7·15 |
| 26 | | 1986 | 2 | 3·81 | 5·85 |
| 27 | | 1986 | 2 | 4·53 | 4·36 |
| 28 | 11/I | 2003 | 1 | 2·32 | – |

[a]Determined by sequencing of ORF2 amplification products.

[b]ORF2 amplification products were determined to be PARV4 genotype 1 sequences, while the amplified variable ORF1 region was determined to be PARV4 genotype 2.

[c]Factor VIII lot tested positive for PARV4 DNA by qualitative PCR but the viral load was below the level of quantification by real-time PCR, and was therefore given an arbitrary viral load of < 2 $log_{10}$ genome copies per ml product.

–, product tested negative for B19V DNA.

### Analysis of PARV4 sequences

Previous analysis of PARV4 sequences showed that ORF1 was slightly less conserved than ORF2 [10]. We therefore amplified and sequenced a 562-bp variable region at the 5′ end of ORF1 from 26/28 factor VIII concentrates testing positive for PARV4 sequences. It had not been possible to amplify the 562-bp variable ORF1 region of PARV4 from factor VIII products 7 and 9 (Table 2). Both PARV4 genotype 1 and 2 sequences were amplified from factor VIII product number 16 (Table 2). Phylogenetic analysis of these PARV4 sequences shows that they fall into two distinct genetic clusters, representing genotypes 1 and 2 (Fig. 3). Across the two genotypes, PARV4 nucleotide sequences amplified from factor VIII products differ from each other by greater than 11% over the region sequenced. Within each genotype, all PARV4 sequences amplified from factor VIII concentrates were greater than 99% homologous (at the nucleotide level, over the 515-bp region sequenced), despite products being manufactured over a 30- to 35-year period. In fact, several PARV4 genotype 1 and 2 sequences amplified from factor VIII products manufactured as early as the mid-1970s were 100% identical at the nucleotide level, over the 515-bp region sequenced, to the recently identified respective strains BR10749 (genotype 1) and BR10627 (genotype 2) [10].

## Discussion

We recently demonstrated the presence of the newly identified human parvovirus PARV4 including the related genotype 2

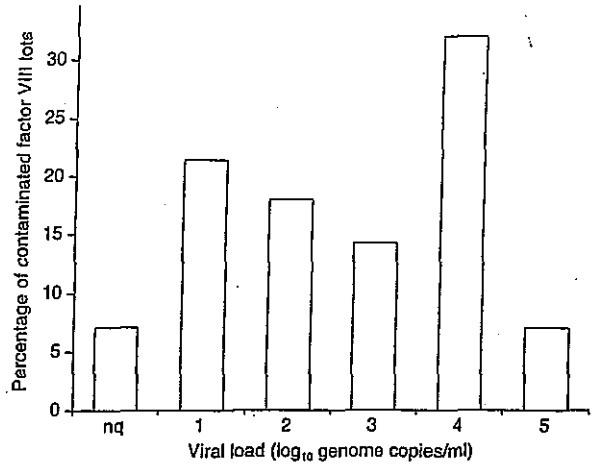


Fig. 2 Viral DNA loads of PARV4 ($log_{10}$ genome copies per ml) in contaminated factor VIII concentrates. nq, not quantifiable.

virus (previously termed PARV5) in manufacturing plasma pools, with these viruses detected in approximately 5% of pools [8,9]. In this present study, we have detected PARV4 viruses in products derived from such plasma pools, specifically in coagulation factor VIII products, manufactured over the past 30–35 years. Information regarding the source of plasma used in the manufacture of products examined in this study was difficult to obtain as it is not provided with the products. These details could only be obtained for the most recent factor VIII product testing positive for PARV4 DNA. This factor VIII concentrate had an expiry date of October 2003, and was manufactured from two plasma pools in September 2000. Donations relating to these plasma pools were collected in or after July 1998 from paid donors from the USA. This suggests that viruses detected in these factor VIII products may date from up to 5 years prior to the expiry date on the product. Details from other manufacturers of recent factor VIII concentrates (testing negative for PARV4) also indicate that donations relating to these products were sourced up to 5 years prior to the expiry date.

The prevalence of PARV4 in factor VIII concentrates was found to be greater in products expiring pre-1990 than in those with an expiry date of post-1990. This difference in the prevalence of PARV4 in factor VIII products over time may reflect the introduction of blood safety measures from the mid-1980s in response to the HIV epidemic, in particular, the introduction of screening tests for HIV and hepatitis C virus (HCV) (in 1986 and 1991, respectively), and virus inactivation of manufacturing plasma pools (introduced in the mid-1980s). The screening of blood donations for HIV and HCV identified 'high-risk' donor groups, such as homosexual males and individuals with a history of intravenous drug use (IVDU), and these groups were subsequently excluded from donating blood [14]. Factor VIII products tested in this study

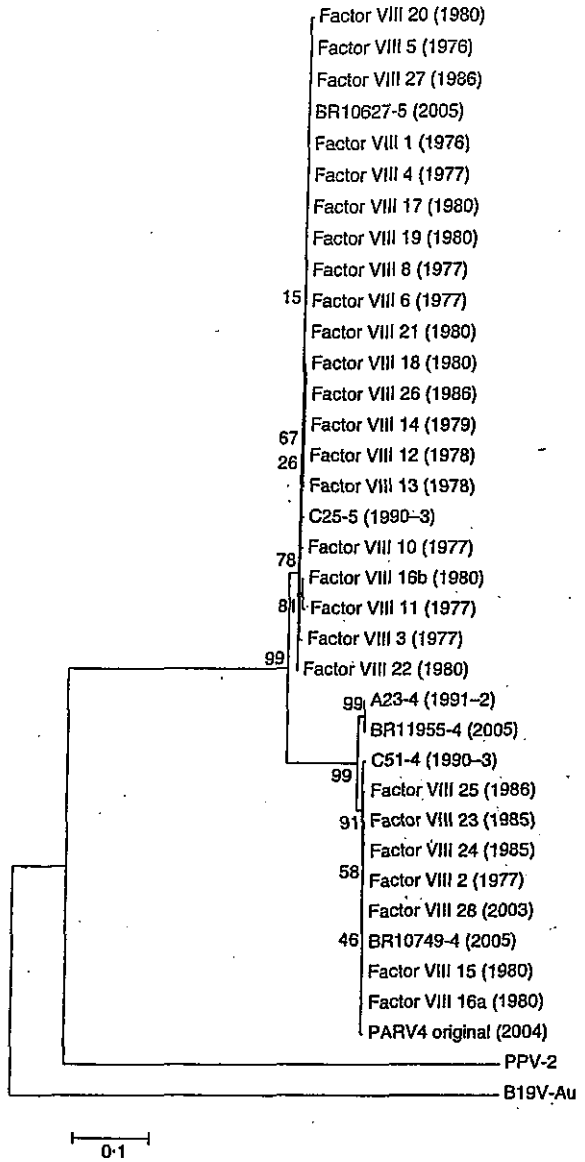


Fig. 3 Phylogenetic analysis of a 515-bp region of ORF1 of PARV4 amplified from factor VIII concentrates. Sequences are named according to factor VIII number and expiry of lot (Table 2). The alignment includes other recently sequenced strains of PARV4 genotype 1; PARV4 original (GenBank accession no. AY622943), BR10749-4 (GenBank accession no. DQ873386), BR11955-4 (GenBank accession no. DQ873388), A23-4 (GenBank accession no. DQ873389) and C51-4 (GenBank accession no. DQ873387); and PARV4 genotype 2, BR10627-5 (GenBank accession no. DQ873390) and C25-5 (GenBank accession no. DQ873391). The PARV4 original strain was sourced from the index case patient in 2004 [1]. Strains BR10749-4 and BR10627-5 were identified in our preliminary study of plasma pools [8], while the other strains were identified in further screening studies of manufacturing plasma pools [9]. Strains BR10749-4, BR11955-4 and BR10627-5 were from plasma samples received at NIBSC between 2004 and 2005, while A23-4, C51-4 and C25-5 were received at NIBSC 1990–1993. The alignment also includes the corresponding nucleotide sequences of porcine parvovirus 2 (PPV-2) (GenBank accession no. AB076669) and B19V-Au genotype 1 virus (GenBank accession no. M13178) as outgroups. Genetic distance and bootstrap values are indicated.

 

with an expiry date of post-1990 are likely to originate from plasma sourced from screened 'low-risk' blood donors. Therefore, the reduced prevalence of PARV4 in more recently manufactured factor VIII products may be a result of the removal of specific 'high-risk' donor populations.

Virus inactivation using a variety of heat treatments was introduced into the manufacturing process of existing coagulation factor products in the mid-1980s, before the implementation of HIV and HCV screening. The effectiveness of these treatments, for HCV particularly, varied greatly, depending on the duration and temperature of heating and whether the product is in liquid form or lyophilized [15,16]. Other virus inactivation procedures include solvent/detergent treatment, which is effective against enveloped viruses [17,18]. Animal parvoviruses, such as canine, bovine and porcine parvoviruses, and minute virus of mice, were used to investigate the effectiveness of virus inactivation of plasma prior to the development of cell culture-based assays for B19V. By virtue of their small size and absence of viral envelope, animal parvoviruses are relatively resistant to inactivation by a range of heat and chemical agents [19]. Based on studies using these model parvoviruses, B19V was also expected to be resistant to these virus inactivation strategies and unlikely to be effectively eliminated by dry heat and pasteurization [5]. However, recent studies using B19V cultures suggest that it is more susceptible to heat and low pH treatments than other animal parvoviruses [20–23]. Results here show that there was not a significant reduction in the prevalence of B19V DNA in factor VIII products manufactured after the introduction of virus inactivation procedures (B19V DNA was detected in 41% of products manufactured without virus inactivation measures vs. 39% of products manufactured using virus inactivation steps). However, it must be noted that virus inactivation procedures such as heat and low pH treatments do not physically remove viral DNA, which may still be detectable by NAT. The effect of virus inactivation procedures on PARV4 remains to be determined; however, the reduced prevalence of PARV4 in factor VIII products manufactured with virus inactivation (8% in virus inactivated products vs. 22% in products manufactured without virus inactivation) may suggest that these viruses are susceptible to virus inactivation treatments. The increased prevalence of PARV4 in factor VIII concentrates expiring in the late 1970s and mid-1980s may also result from epidemics of infection as has been observed for B19V [2]. Our investigation of recent and archived manufacturing plasma pools for PARV4 identified an increased prevalence of these viruses in plasma pools received from one manufacturer between 1991 and 1992, which may be the result of seasonal and/or epidemic variation [9].

PARV4 viral loads in these factor VIII concentrates were as high as 5 $\log_{10}$ per ml of product, while the levels of B19V were as high as 8 $\log_{10}$ per ml of product. The higher levels of contaminating PARV4 and B19V viruses were confined to the older factor VIII concentrates (expiring pre-1990). Considering that downstream purification and processing of manufacturing plasma pools will alter the viral loads present in subsequent plasma-derived products, viral loads in these factor VIII concentrates correlate well, albeit being approximately 1 $\log_{10}$ lower, with the levels of PARV4 and B19V detected in recent and archived plasma pools [8,9]. In these manufacturing plasma pools, the viral loads of these viruses typically range up to 6 $\log_{10}$ per ml of plasma for PARV4, and up to 9 $\log_{10}$ per ml of plasma for B19V.

In manufacturing plasma pool samples previously examined for the presence of PARV4, we found that genotypes 1 and 2 were detected in approximately equal proportions [8,9]. These samples were received at NIBSC for plasma pool testing between 2005 and 2006, but also included archived samples received between 1990 and 1993. In this present study, we detected a greater prevalence of PARV4 genotype 2 over genotype 1 in factor VIII concentrates manufactured in the past 30–35 years (21 products testing positive for PARV4 genotype 2 sequences vs. nine products testing positive for PARV4 genotype 1 sequences). As the majority of these PARV4-positive factor VIII products had expiry dates of pre-1990 and were likely to have been manufactured from blood donations collected before the mid-1980s, these results suggest a temporal change in the prevalence of PARV4 genotypes over the past 30–35 years. A similar temporal change in parvovirus genoprevalence has been suggested in the case of B19V genotypes 1 and 2, where both genotypes were equally detected in the tissues of individuals born in the 1950s or earlier, while genotype 1 viruses were predominantly detected in the tissues of individuals born in the 1960s and later [24]. Further evidence for a temporal succession of infection with PARV4 genotype 1 over genotype 2 has recently been reported in HIV infected patients [25].

Although positive PCR results do not necessarily reflect infectivity, the detection of PARV4 DNA in coagulation factor VIII concentrates in this study raises questions as to whether PARV4 has been transmitted parenterally to the recipients of such products. PARV4 was originally identified in an individual who was a daily injecting drug user and it is possible that he acquired the virus through this route [1]. In addition, we have identified an increased incidence in the detection of PARV4 in febrile patients, including IVDUs and homosexual men [9], and in individuals infected with HCV (including IVDUs) [26]. An increased prevalence of PARV4 in HIV-infected individuals has also recently been reported [25]. Nothing is yet known as to whether there is any pathology associated with PARV4 infection. Although the PARV4 index case patient had an acute viral infection syndrome, the lifestyle of this individual and an underlying infection with HBV make it impossible to determine whether PARV4 played a role in his symptoms [1]. The presence of PARV4 in pooled plasma from healthy blood donors suggests that these viruses

may cause subclinical infections, and the implications for the safety of blood and plasma-derived products such as factor VIII are still not known.

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医薬品　研究報告　調査報告書

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| 販売名（企業名） | 別紙のとおり | | | | |

**研究報告の概要**

問題点：脳出血で死亡した臓器提供者から腎臓と肝臓の提供を受けた後に死亡した3人の女性から発見されたウイルスは、遺伝子配列解析により、リンパ球性脈絡膜髄膜炎ウイルス様の新種のアレナウイルスと判明した。

2006年12月に3ヵ月間の前ユーゴスラビアの地方滞在からオーストラリアに帰国して10日後に脳出血で死亡した57才の臓器提供者から、腎臓と肝臓の提供を受けた63,64,44才の3人の女性が死亡した。女性たちは、移植直後の経過に変わったところはなかったが、その後脳症を伴う熱性疾患を発症し、提供を受けて4～6週後の2007年1月初めに死亡した。2人の患者から移植されたそれぞれの肝臓と腎臓のRNAの塩基配列を解析した結果、リンパ球性脈絡膜髄膜炎ウイルス（LCMV）様の新種のアレナウイルスと思われる遺伝子配列が検出された。また、PCR解析により、患者の腎臓、肝臓、血液及び髄液からウイルスの遺伝子断片が、免疫組織化学的解析により、移植された肝臓及び腎臓からウイルスの抗原が検出された。さらに、患者血清からは抗ウイルスIgM及びIgG抗体も検出された。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 別紙のとおり | 今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。 |

使用上の注意記載状況・その他参考事項等

記載なし

| | |
|---|---|
| 一般的名称 | ①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ |
| 販売名(企業名) | ①献血アルブミン 20“化血研”、②献血アルブミン 25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500 注射用 |
| 報告企業の意見 | LCMV は、アレナウイルス属に属するエンベロープに包まれた直径 30～300nm の不定形粒子であり、二種類のマイナス一本鎖 RNA を有する。げっ歯類を自然宿主とし、その糞尿や唾液、血液の曝露によってヒトに伝播する。LCMV 感染症は多くは無症候性あるいは軽度であるが、妊婦では無菌性髄膜炎や脳炎、致死性の感染症を起こす危険がある。臓器移植患者における LCMV のヒト-ヒト感染は、過去にも報告がある。<br>弊所の血漿分画製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。<br>各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第 1047 号、平成 11 年 8 月 30 日)」に従い、ウシウイルス性下痢ウイルス（BVDV)、仮性狂犬病ウイルス（PRV)、ブタパルボウイルス（PPV)、A 型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告したリンパ球性脈絡膜髄膜炎ウイルス（LCMV）は、エンベロープの有無、核酸の種類等からモデルウイルスとしては BVDV が該当すると考えられるが、上記バリデーションの結果から、BVDV の除去・不活化効果を有することを確認している。<br>また、これまでに当該製剤による LCMV 感染の報告例は無い。<br>以上の点から、当該製剤は LCMV に対する安全性を確保していると考える。 |

*現在製造を行っていない

The NEW ENGLAND JOURNAL of MEDICINE

# A New Arenavirus in a Cluster of Fatal Transplant-Associated Diseases

Gustavo Palacios, Ph.D., Julian Druce, Ph.D., Lei Du, Ph.D., Thomas Tran, Ph.D., Chris Birch, Ph.D., Thomas Briese, Ph.D., Sean Conlan, Ph.D., Phenix-Lan Quan, Ph.D., Jeffrey Hui, B.Sc., John Marshall, Ph.D., Jan Fredrik Simons, Ph.D., Michael Egholm, Ph.D., Christopher D. Paddock, M.D., M.P.H.T.M., Wun-Ju Shieh, M.D., Ph.D., M.P.H., Cynthia S. Goldsmith, M.G.S., Sherif R. Zaki, M.D., Ph.D., Mike Catton, M.D., and W. Ian Lipkin, M.D.

## ABSTRACT

### BACKGROUND

Three patients who received visceral-organ transplants from a single donor on the same day died of a febrile illness 4 to 6 weeks after transplantation. Culture, polymerase-chain-reaction (PCR) and serologic assays, and oligonucleotide microarray analysis for a wide range of infectious agents were not informative.

### METHODS

We evaluated RNA obtained from the liver and kidney transplants in two recipients. Unbiased high-throughput sequencing was used to identify microbial sequences not found by means of other methods. The specificity of sequences for a new candidate pathogen was confirmed by means of culture and by means of PCR, immunohistochemical, and serologic analyses.

### RESULTS

High-throughput sequencing yielded 103,632 sequences, of which 14 represented an Old World arenavirus. Additional sequence analysis showed that this new arenavirus was related to lymphocytic choriomeningitis viruses. Specific PCR assays based on a unique sequence confirmed the presence of the virus in the kidneys, liver, blood, and cerebrospinal fluid of the recipients. Immunohistochemical analysis revealed arenavirus antigen in the liver and kidney transplants in the recipients. IgM and IgG antiviral antibodies were detected in the serum of the donor. Seroconversion was evident in serum specimens obtained from one recipient at two time points.

### CONCLUSIONS

Unbiased high-throughput sequencing is a powerful tool for the discovery of pathogens. The use of this method during an outbreak of disease facilitated the identification of a new arenavirus transmitted through solid-organ transplantation.

From the Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York (G.P., T.B., S.C., P.-L.Q., J.H., W.I.L.); Victorian Infectious Diseases Reference Laboratory, Victoria, Australia (J.D., T.T., C.B., J.M., M.C.); 454 Life Sciences, Branford, CT (L.D., J.F.S., M.E.); and the Centers for Disease Control and Prevention, Atlanta (C.D.P., W.-J.S., C.S.G., S.R.Z.). Address reprint requests to Dr. Lipkin at the Center for Infection and Immunity, Mailman School of Public Health, Columbia University, 722 W. 168th St., New York, NY 10032, or at wil2001@columbia.edu, or to Dr. Catton at the Victorian Infectious Diseases Reference Laboratory, Locked Bag 815, Carlton South, Victoria 3053, Australia, or at mike.catton@mh.org.au.

Drs. Palacios and Druce contributed equally to this article.

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METHODS OF CLONING NUCLEIC ACIDS of microbial agents directly from clinical specimens offer new opportunities for the surveillance and discovery of pathogens. Molecular techniques have been used successfully in the identification of infectious agents such as the Borna disease virus, hepatitis C virus, Sin Nombre virus, human herpesviruses 6 and 8, *Bartonella henselae*, *Tropheryma whipplei*, West Nile virus, and the coronavirus associated with severe acute respiratory syndrome.[1]

The arenaviruses are enveloped, negative-strand RNA viruses in rodents; these viruses are most frequently transmitted to humans through exposure to infected urine. Infection with the prototype virus, lymphocytic choriomeningitis virus (LCMV), is typically asymptomatic or associated with mild, transient illness; however, LCMV has also been implicated in aseptic meningitis.[2] Human-to-human transmission of LCMV during pregnancy has been reported, and infection during the gestational period can result in fetal death, neurologic sequelae, and chorioretinopathy.[3] Fatal outbreaks of disease associated with human-to-human transmission of LCMV in recipients of solid-organ transplants have also been described.[4] We report the use of unbiased DNA sequencing in the discovery of a new LCMV-related arenavirus that caused fatal disease in three recipients of organs from a single donor.

## METHODS

### PATIENTS AND CLINICAL COURSE

Three women in Australia who were 63 years of age (Recipient 1), 64 years of age (Recipient 2), and 44 years of age (Recipient 3) received a liver transplant (Recipient 2) or kidney transplants (Recipients 1 and 3) from one male donor who was 57 years of age. The donor died of cerebral hemorrhage 10 days after returning to Australia from a 3-month visit to the former Yugoslavia, where he had traveled in rural areas. The immediate post-transplantation course in the three transplant recipients was unremarkable; however, febrile illnesses with varying degrees of encephalopathy developed in all three, and they died 4 to 6 weeks after transplantation (Table 1). Bacterial and viral cultures; polymerase-chain-reaction (PCR) assays for herpesviruses 1 through 8, lyssavirus, influenza A and B viruses, respiratory syncytial virus, picornavirus, adenovirus, human parainfluenza virus, flavivirus, alphavirus, hantavirus, polyomavirus, Crimean–Congo hemorrhagic fever virus, Rift Valley fever virus, toxoplasma, *Mycobacterium tuberculosis*, and *Mycoplasma pneumoniae*; and viral and panmicrobial oligonucleotide microarray analysis[4] revealed no candidate pathogens.

### UNBIASED HIGH-THROUGHPUT SEQUENCING

RNA was extracted from the brain, cerebrospinal fluid, serum, kidney, and liver of Recipient 1, who had received a kidney transplant, and from the cerebrospinal fluid and serum of Recipient 2, who had received a liver transplant. As shown in Figure 1, after digestion with DNase I to eliminate human chromosomal DNA, RNA preparations were amplified by means of reverse-transcriptase PCR (RT-PCR) with the use of random primers.[5,6] Amplification products were pooled and sequenced with the use of the GSL FLX platform (454 Life Sciences), but DNA fragmentation was omitted.[7] After trimming to remove sequences derived from the amplification primer and after filtration to eliminate highly repetitive sequences, the data set was analyzed by subtracting fragments that matched human sequences, clustering nonredundant sequences,[8] and assembling them into contiguous sequences[9] for direct comparison with the GenBank databases of nucleic acids and proteins with the use of BLASTN and BLASTX software.[10] We analyzed the resulting alignments and assigned them to nodes in the National Center for Biotechnology Information taxonomy database, using a custom software application written in Perl (BioPerl version 5.8.5).

### PCR QUANTITATION OF THE ARENAVIRUS BURDEN

RNA obtained from tissues, plasma, serum, and cerebrospinal fluid was reverse transcribed with the use of random hexamers. PCR was performed with the use of a SYBR Green assay (Applied Biosystems). The following cycling conditions were used: 50°C for 2 minutes and 95°C for 10 minutes, followed by 45 cycles at 95°C for 15 seconds and 60°C for 1 minute. Real-time PCR assays were performed with the following primer set: 5′AGTGCYTGCACAACATCGTTT3′ (forward) and 5′CAATGCCCAGCYTGACAAT3′ (reverse). Thermal cycling was performed with the use of an ABI 7500 real-time PCR system (Applied Biosystems).

Table 1. Characteristics of the Organ-Transplant Recipients.

| Recipient No. | Age | Diagnosis | Organ Transplanted | Clinical Course | Interval between Transplantation and Death |
|---|---|---|---|---|---|
| | *yr* | | | | *days* |
| 1 | 63 | End-stage renal failure due to polycystic kidney disease | Kidney | Fever, sepsis, encephalopathy, acute tubular necrosis, graft rejection, radiographic evidence of chest infiltrates | 36 |
| 2 | 64 | Decompensated cirrhosis and hepatocellular cancer due to hepatitis C infection | Liver | Fever, confusion, encephalopathy with myoclonus, chest infiltrates | 30 |
| 3 | 44 | End-stage renal failure due to polycystic kidney disease | Kidney | Fever, graft rejection, intraabdominal hematomas and effusion, transplant nephrectomy, encephalopathic illness | 29 |

### VIRAL ISOLATION AND ANALYSES

Kidney tissue from Recipient 1 was homogenized in phosphate-buffered saline, centrifuged to pellet cellular debris, filtered, and used to inoculate Vero E6 cells. The cells were monitored daily by means of light microscopy for cytopathic effect and by means of RT-PCR for the presence of arenavirus nucleic acid in supernatant. Monolayers of cells showing cytopathic effects that were also positive for arenavirus nucleic acid were fixed with buffered 4% paraformaldehyde for indirect immunofluorescence and immunohistochemical microscopy and with buffered 2.5% glutaraldehyde for thin-section electron microscopy. Rabbit polyclonal antiserum against Old World arenaviruses, including LCMV, was used as the source of primary antibodies for immunohistochemical analysis. Secondary antibodies were alkaline phosphatase–conjugated goat antibodies against rabbit IgG.[4] Immunohistochemical assays were also performed with the use of formalin-fixed, paraffin-embedded tissue sections obtained from the liver and kidney of Recipient 1.

Virus-infected and noninfected (control) Vero E6 cells were fixed with methanol. Serum specimens from the donor, from the recipients, and from 100 randomly chosen control recipients of solid-organ transplants were applied to the fixed cells followed by fluorescein-labeled antihuman IgG or IgM secondary antibodies.

### COMPLETE GENOME SEQUENCING AND PHYLOGENETIC ANALYSES

RNA extracted from the liver in Recipient 1 was used as a template to clone and sequence the L and S segments of the virus. The gene fragments obtained by means of pyrosequencing were used to design specific PCR primers; thereafter, consensus primers were designed on the basis of alignments of other arenavirus sequences with the use of the SCPrimer program.[11]

The L and S segments were assembled and sequenced as a series of overlapping genetic fragments. Evolutionary distances between the assembled segments were computed with the use of the Poisson correction method and expressed in units of amino acid substitutions per site in relationship to arenavirus L, glycoprotein precursor, and nucleoprotein amino acid segments in the GenBank database with the use of the MEGA program.[12] The percentage of replicate trees in which taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (see Fig. 1a, 1b, and 1c of the Supplementary Appendix, available with the full text of this article at www.nejm.org). The nucleotide and amino acid homologies of each of the arenavirus genes (Z, L, GPC, and NP) to LCMV (the closest completely sequenced relative) are shown in Table 2. The sequences are deposited in GenBank (accession numbers EU136038 and EU136039).

## RESULTS

RNA from tissue from Recipient 2, who had received a liver, and Recipient 1, who had received a kidney, was pooled and amplified for unbiased high-throughput sequencing,[7] yielding 103,632 se-

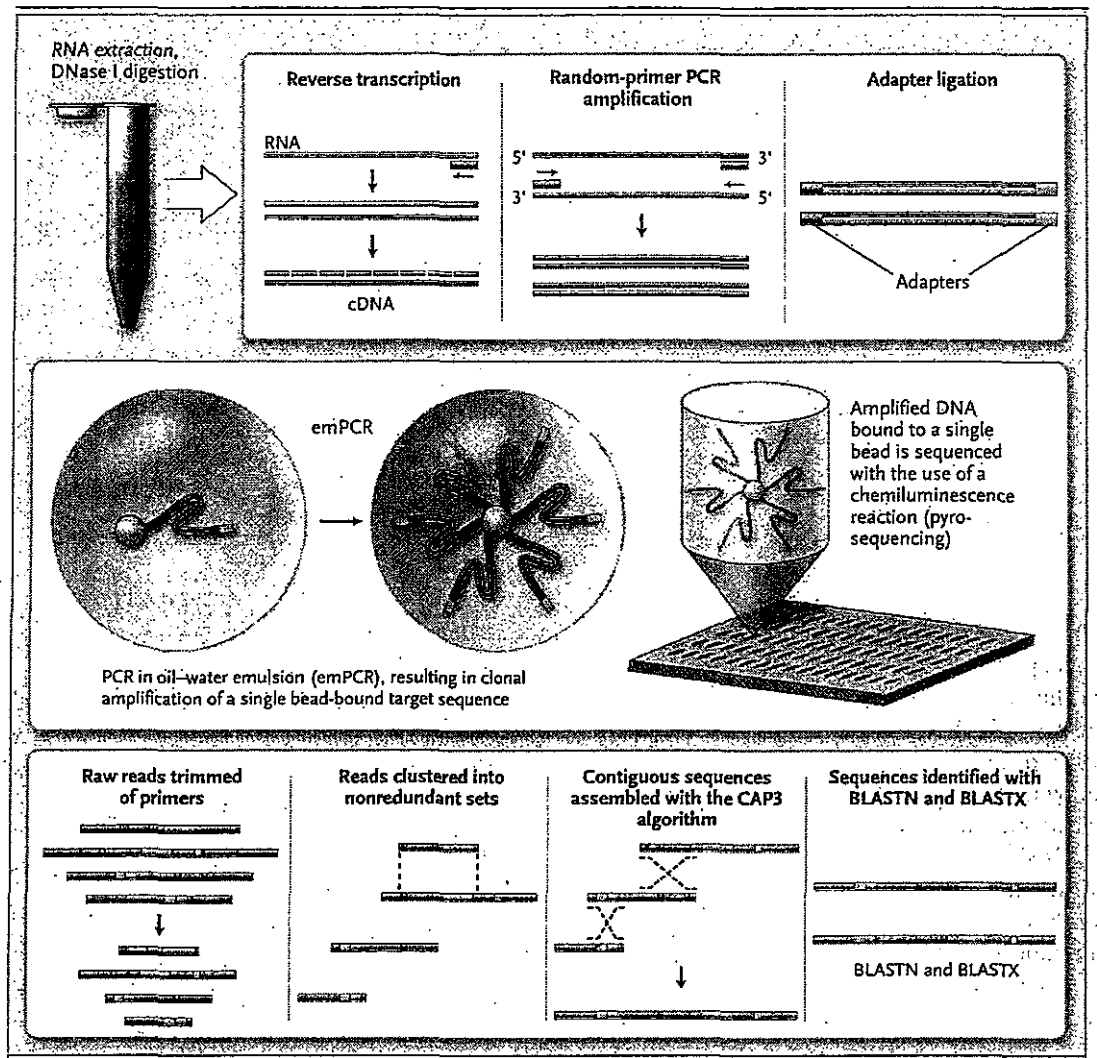


**Figure 1. High-Throughput Sequencing Method.**
PCR denotes polymerase chain reaction.

quence fragments. The sequences recovered ranged in size from 45 to 337 nucleotides, with a mean length of 162. Sequences derived from the amplification primer and highly repetitive sequences were eliminated, yielding a net of 94,043 sequences. These sequences were processed with the use of algorithms that subtract vertebrate sequences, assemble contiguous sequences, and compare the residual nucleotide and deduced amino acid sequences in all six potential open reading frames with motifs represented in databases of microbes.

At the nucleotide level, sequence data were uninformative; however, BLASTX analysis of the deduced protein sequence revealed 14 fragments that were consistent with Old World arenaviruses (12 S-segment and 2 L-segment fragments) sharing the closest relationship to LCMV.

Primers were designed for RT-PCR experiments to detect viral RNA in clinical specimens, assess the similarity of viral sequences among individual organs and recipients, and extend the viral sequence needed to facilitate characterization. Viral RNA was present in a total of 22 of 30 specimens of tissue, blood, or cerebrospinal fluid from all three transplant recipients (Table 3). The sequence was identical in all specimens, a finding that was consistent with the introduction of a single virus into all the recipients.

Fresh-frozen kidney tissue from Recipient 1 was homogenized and used to inoculate cultures of

Vero E6 cells. A cytopathic effect was observed only in the first passages; thereafter, morphologic characteristics did not differ between infected and control cells. Indirect immunofluorescence assays with the use of polyclonal antibodies against arenaviruses and LCMV showed cytoplasmic distribution of viral antigen. Immunostaining of viral antigens was also seen in infected cells by means of an indirect immunoalkaline phosphatase technique (Fig. 2A). Quantitative RT-PCR assays showed increasing concentrations of viral nucleic acid with serial passage. Examination of infected Vero E6 cells by means of thin-section electron microscopy revealed extracellular particles with morphologic features that are characteristic of arenaviruses (Fig. 2B).

Immunofluorescence assays for serum antibodies that are reactive with infected Vero E6 cells revealed virus-specific IgM and IgG antibodies in the donor that were consistent with acute infection. Plasma and serum specimens from Recipient 2 that had been collected at two time points 19 days apart (11 days and 30 days after transplantation) were available for analysis. Virus-specific IgG and IgM antibodies were detectable only at the second time point, consistent with seroconversion.

Immunohistochemical analysis of specimens of the liver (Fig. 3A) and kidney (Fig. 3B) obtained from Recipient 1 showed focal immunostaining of arenavirus antigens. PCR surveys of 100 archived serum or plasma specimens from solid-organ transplant recipients who were not linked to the cluster and who had undergone transplantation in the same city and during the same time period revealed no evidence of infection with this pathogen.

The 3301-nucleotide S-segment and 7215-nucleotide L-segment sequences were cloned from the kidney of Recipient 1 by means of PCR and sequenced. Phylogenetic characterization was limited by the paucity of available sequences deposited in public databases; nonetheless, L- and S-segment analyses were consistent with the presence of a new arenavirus. Whereas sequences in the nucleoprotein and glycoprotein genes on the S segment were closest to the LCMV strain LE[13] and M1 and M2 isolates[14] (Table 2, and Fig. 1b and 1c of the Supplementary Appendix), the L-segment sequence indicated a closer relationship to Kodoko virus. Strain LE was isolated in France from an infected fetus. M1 and M2 were isolated in Japan from wild mice. Kodoko virus was recently isolated in Africa from wild mice (Fig. 1a of the Supplementary Appendix).[15] Reassortment is well described in arenaviruses and could account for differences in phylogenetic relationships based on L- and S-segment sequences. However, reassortment cannot be implicated without a complete genomic sequence for the viruses used in these phylogenetic analyses.

**Table 2. Nucleotide and Amino Acid Homologies of the New Arenavirus to Other Arenaviruses.***

| Gene | Accession No. | LCMV Strain | Homology | |
|---|---|---|---|---|
| | | | Amino Acid | Nucleotide |
| | | | *percent* | |
| GPC | AB261990 | M2 | 94 | 86 |
| NP | AB261990 | M2 | 97 | 87 |
| L | DQ286932 | Marseille 12 | 82 | 79 |
| Z | DQ286932 | Marseille 12 | 79 | 72 |

* LCMV denotes lymphocytic choriomeningitis virus.

## DISCUSSION

Two clusters of transmission of arenavirus through solid-organ transplantation have been reported.[4] In each cluster, recipients linked to a single donor died of an unexplained infectious disease 9 to 76 days after transplantation. In neither cluster did the donor have a history of acute infectious disease or evidence of infection by PCR or serologic analysis; however, in one cluster, a pet hamster that had recently been introduced into the donor's household was found to be infected with the same virus that was detected in the recipients. LCMV was implicated after the results of viral culture and electron microscopy triggered specific immunohistochemical and molecular tests for arenaviruses.

In our cluster, a new arenavirus was first detected through unbiased high-throughput sequencing. Thereafter, the infection was confirmed by means of culture, electron microscopy, and specific immunohistochemical and serologic tests. As in the other two reported clusters of transplant-associated transmission, we detected no viral nucleic acids in the donor and found no history of acute infectious disease; however, the presence of IgG and IgM antibodies confirmed recent infection. We were also unable to obtain any infor-

Table 3. Viral RNA and Antibody Titers in the Donor and Recipients.*

| Specimen | Interval between Transplantation and Collection of Specimens | Viral RNA | Antibody Titer |
|---|---|---|---|
| | *days* | *copies/ml of RNA extract* | |
| Donor | | | |
| Serum | 0 | ND | 1:80 IgG, 1:20 IgM |
| Spleen | 0 | ND | NA |
| Pancreas | 0 | ND | NA |
| Recipient 1 (kidney transplant) | | | |
| Plasma | 0 | ND | <1:10 IgG, <1:10 IgM |
| Plasma | 27 | 889,200 | NP |
| Plasma | 33 | 614,900 | NP |
| Cerebrospinal fluid | 33 | 5,500 | NP |
| Plasma | 35† | 1,000,000 | NP |
| Urine | 35† | 88,000,000 | NA |
| Heart | 35† | 33,200 | NA |
| Spleen | 35† | 52,600 | NA |
| Liver | 35† | 2,362,800 | NA |
| Lung | 35† | 498,600 | NA |
| Cerebrospinal fluid | 35† | 63,700 | NP |
| Serum | 35† | 1,440,400 | <1:10 IgG, <1:10 IgM |
| Brain | 35† | 16,600 | NA |
| Rectal swab | 35† | 623,200 | NA |
| Nasal swab | 35† | 55,400 | NA |
| Axillary swab | 35† | ND | NA |
| Kidney | 35† | 85,900 | NA |
| Recipient 2 (liver transplant) | | | |
| Plasma | 12 | 121,900 | <1:10 IgG, <1:10 IgM |
| Mouth swab | 24 | 457,000 | NA |
| Bronchoalveolar lavage | 19 | 1,163,400 | NA |
| Cerebrospinal fluid | 24 | ND | NP |
| Plasma | 24 | 346,200 | NP |
| Serum | 31† | 347,600 | 1:40 IgG, 1:20 IgM |
| Recipient 3 (kidney transplant) | | | |
| Serum | −235 | ND | <1:10 IgG, <1:10 IgM |
| Serum | 0 | ND | NP |
| Serum | 24 | 415,500 | NP |
| Serum | 28 | 565,100 | <1:10 IgG, <1:10 IgM |

* NA denotes not applicable, ND not detected, and NP not performed.
† Specimens were obtained after death.

mation indicating that the donor had been exposed to rodents; however, his history of recent travel suggests that he may have been infected before returning to Australia from southern Europe, where such exposure may have occurred in a rural area.

Although we have not fulfilled Koch's postulates, evidence implicating this new virus in the

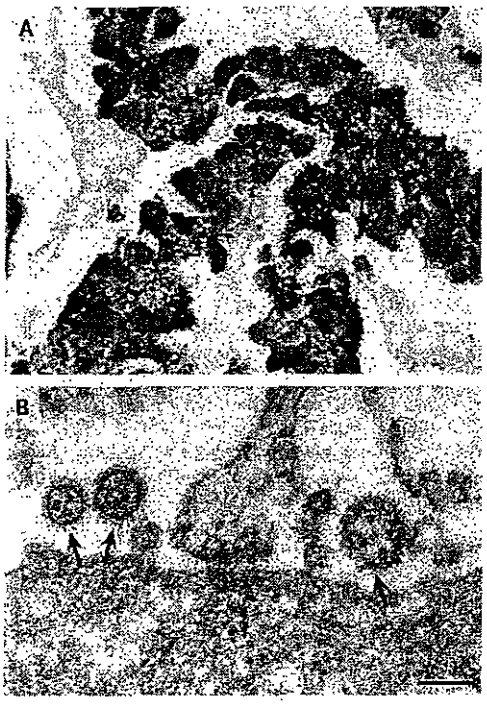


**Figure 2. Propagation of the New Arenavirus in Tissue Culture.**

Panel A shows immunostaining of viral antigens in infected cells by means of an indirect immunoalkaline phosphatase technique. Panel B shows an electron micrograph of extracellular arenavirus-like virions. Particles (arrows) are round, vary in size, and have surface projections on the perimeter. Cellular ribosomes are visible within the virions. The length of the bar corresponds to 100 nm.

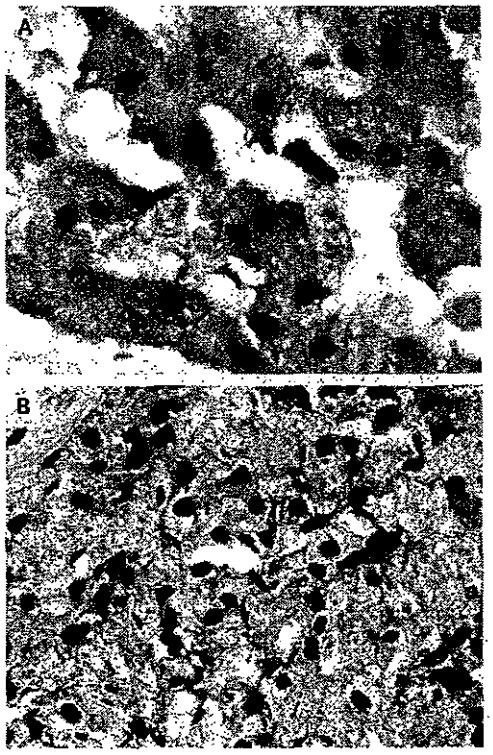


**Figure 3. Predominantly Membranous Distribution of Arenavirus Antigen.**

The distribution of the arenavirus antigen is shown in the liver (Panel A) and kidney (Panel B) of Recipient 1. Formalin-fixed, paraffin-embedded tissue sections were incubated with polyclonal rabbit antiserum against lymphocytic choriomeningitis virus followed by alkaline phosphatase–conjugated secondary antibodies against rabbit IgG.

outbreak of infection among patients who received transplants is compelling. All three recipients received organs from the same donor and died within days of one another after febrile illness. Identical viral sequences were obtained from all the recipients. The virus is new and was not detected in 100 organ recipients who were not linked to this cluster. The results of serologic analysis of specimens obtained from the donor were consistent with recent infection, and seroconversion was observed in one recipient.

Unbiased high-throughput sequencing has been used to characterize complex mixtures of microflora in environmental contexts[16]; we have shown that this strategy can be used to address a suspected outbreak of infectious disease. Its use in the context of investigating a cluster of cases of acute disease associated with organ transplantation facilitated the rapid implication of a new arenavirus not detected by other methods. This technique may prove useful as a new tool in the identification and surveillance of pathogens in chronic as well as acute disease.

Supported by grants from the National Institutes of Health (U54AI57158, to the Northeast Biodefense Center–Lipkin; U01AI070411; AI062705; and HL083850).

Drs. Du, Simons, and Egholm report being employees of 454 Life Sciences. Dr. Lipkin reports being a member of the scientific advisory board of 454 Life Sciences during a portion of the time the work reported here was pursued. Drs. Du, Simons, Egholm, and Lipkin report holding stock options in 454 Life Sciences before it was purchased by Roche Diagnostics in May 2007. No other potential conflict of interest relevant to this article was reported.

We thank David Riches for skillful technical assistance.

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別紙様式第2-1

No. 26

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
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| 一般的名称 | 人赤血球濃厚液 | 研究報告の公表状況 | | 公表国 |
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| 販売名(企業名) | 赤血球濃厚液-LR「日赤」(日本赤十字社)<br>照射赤血球濃厚液-LR「日赤」(日本赤十字社) | | 岡田義昭, 水澤左衛子. 2007年プリオン研究会 | 日本 |

**研究報告の概要**

○BSE由来プリオンのin vitro感染系の確立とその応用

目的:輸血によるvCJD感染が英国で4例報告され、分画製剤を含めた血液製剤の安全性確保が重大な課題になっている。しかし、除去評価の対象となる検体や除去効果を評価する適当な方法がないのが現状である。我々は血液を対象としているため、さらに血液中にどのような様式で$PrP^{res}$が存在するのか、明らかにする必要がある。そこで我々は、BSE感染ウシ由来の脳乳剤を用いて$PrP^{res}$のin vitro感染系の確立を試みたので報告する。

方法:BSE感染ウシ由来の脳乳剤をマウス及びヒト由来の神経系細胞株、及び血液由来細胞株に添加し、継代しながら経時的にウエスタンブロット法(WB)を行い$PrP^{res}$の有無を検討した。$PrP^{res}$は、核成分を除いた細胞溶解液をPK20 μg/mL、37℃、1時間処理後、メタノール沈殿によって抽出し、ウサギ抗プリオン抗体を用いたWBにて検出した。また、継代した感染細胞の培養上清を段階希釈し、非感染細胞に感染させ、$PrP^{res}$が伝達されるか検討した。さらに、20nmのウイルス除去膜を用いて$PrP^{res}$の除去が可能か検討した。

結果:ヒト由来グリオーマ細胞株から30kD付近にPK耐性で抗プリオン抗体に反応する2本のバンドが検出された。このバンドは非感染細胞には存在しなかった。30kD付近の2本のバンドは感染後10週前後からWBによって検出可能になり、14週頃まで検出された(細胞によっては20～25週頃まで検出することもできた)。また、9ヵ月継代した感染細胞の培養上清を非感染細胞に感染させたところ、30kD付近にPK耐性の2本のバンドが検出され、伝達性があることが明らかになった。さらに20nmのウイルス除去膜を用いて培養上清を濾過したところ、無処理に比較して感染価は約5Log減少し、ウイルス除去膜によって伝達性が減少することが認められた。

考察:BSE由来の$PrP^{res}$を感染させた細胞から抗プリオン抗体に反応する約30kDのPK耐性のバンドが検出された。培養上清によって同様のバンドが非感染細胞に伝達されたことから、脳乳剤から検出される$PrP^{res}$とはバンドのパターンは異なるもののin vitroにおいてBSEの感染が成立したと考えられた。

**使用上の注意記載状況・その他参考事項等**

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| BSE感染ウシ由来の脳乳剤を用いた$PrP^{res}$のin vitro感染系の確立を試みたところ、9ヵ月継代した感染細胞の培養上清に伝達性があることが明らかになった。また、20nmのウイルス除去膜によって培養上清の伝達性が減少することが認められたとの報告である。 | 今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。 |

(5)

JRC2007T-080

# BSE 由来プリオンの in vitro 感染系の確立とその応用

○岡田義昭、水澤左衛子　　国立感染症研究所　血液・安全性研究部

（目的）輸血によるｖＣＪＤ（variant Creutzfelt Jacob Disease）感染が英国で 4 例報告され、分画製剤を含めた血液製剤の安全性確保が重大な課題になっている。しかし、除去評価の対象となる検体や除去効果を評価する適当な方法がないのが現状である。我々は血液を対象としているため、さらに血液中にどのような様式で $PrP^{res}$ が存在するのか、明らかにする必要がある。そこで我々は、BSE 感染ウシ由来の脳乳剤を用いて $PrP^{res}$ の in vitro 感染系の確立を試みたので報告する。

（方法）BSE 感染ウシ由来の脳乳剤をマウス及びヒト由来の神経系細胞株、及び血液由来細胞株に添加し、継代しながら経時的 にウエスタンブロット法（以下ＷＢ）を行い $PrP^{res}$ の有無を検討した。$PrP^{res}$ は、核成分を除いた細胞溶解液をＰＫ20μg/mL、37℃、1 時間処理後、メタノール沈殿によって抽出し、ウサギ抗プリオン抗体を用いたＷＢにて検出した。また、継代した感染細胞の培養上清を段階希釈し、非感染細胞に感染させ、$PrP^{res}$ が伝達されるか検討した。さらに、20ｎｍのウイルス除去膜を用いて $PrP^{res}$ の除去が可能か検討した。

（結果）ヒト由来グリオーマ細胞株から 30Ｋｄ付近に PK 耐性で抗プリオン抗体に反応する 2 本のバンドが検出された。このバンドは非感染細胞には存在しなかった。３０Ｋｄ付近の 2 本のバンドは感染後 10 週前後からＷＢによって検出可能になり、14 週頃まで検出された（細胞によっては 20～25 週頃まで検出することもできた）。また、9 ヶ月継代した感染細胞の培養上清を非感染細胞に感染させたところ、30Ｋｄ付近に PK 耐性の 2 本のバンドが検出され、伝達性があることが明らかになった。さらに 20ｎｍのウイルス除去膜を用いて培養上清を濾過したところ、無処理に比較して感染価は 約 5Log 減少し、ウイルス除去膜によって伝達性が減少することが認められた。

（考察）BSE 由来の $PrP^{res}$ を感染させた細胞から抗プリオン抗体に反応する約 30Ｋの ＰＫ耐性のバンドが検出された。培養上清によって同様のバンドが非感染細胞に伝達されたことから、脳乳剤から検出される $PrP^{res}$ とはバンドのパターンは異なるものの in vitro において BSE の感染が成立したと考えられた。

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
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| | | | | 2007. 9. 20 | 該当なし | |

| 一般的名称 | 抗HBs人免疫グロブリン | 研究報告の公表状況 | | 公表国 |
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| 販売名(企業名) | 抗HBs人免疫グロブリン「日赤」(日本赤十字社) | | Foster P. Prion 2007; 2007 Sep 26-28; Edinburgh. | 英国 |

**研究報告の概要**

○vCJDはヒト血漿製剤によって伝播したのか？20年間と集計
vCJDと診断された人の血漿が、診断以前に英国国立健康増進局(NHS)によって血液製剤の製造に使用されていたため、予防措置として、英国の供血者由来の血漿からの血漿由来製剤の製造中止が1998年に決定された。これ以来、血漿分画製剤は、NHSによって米国及びヨーロッパから購入した血漿を使用して製造されるか、営利企業から直接購入されてきた。
後にvCJDと診断された11人の供血者由来の供血が、1987年6月から1998年9月にかけて出荷された175バッチの様々な血漿分画製剤の原料に含まれていたということが知られている。最初の製品出荷から20年が経過したにもかかわらず、これらの製剤に関連したvCJD症例は発生していない。このことは、赤血球によるvCJD伝播の可能性を示す症例が、輸血後6.5年、7.8年、8.3年で発症したことと対照的である。
血漿分画製剤による明らかな伝播が見られないことについては幾つかの説明がなされている。
(1)供血された血漿中にプリオンの感染性がない
(2)供血された血漿中にプリオンの感染性はあるが、製造工程の希釈や感染性の低減によって、製品には感染性がない
(3)製品にプリオンの感染性はあるが、潜伏期間が長いあるいは投与された患者に感受性がないため、まだ発症していない
プリオン除去の範囲を特定するためスコットランド輸血サービスで血漿分画製剤の製造に用いられている方法を検討した。これらの実験は、プリオン除去能は全体として、中間純度の第Ⅷ因子濃縮製剤で2.7log、中間純度の第Ⅸ因子濃縮製剤で3.0log、トロンビンで5.8log、フィブリノゲンで6.2log以上、免疫グロブリンで6.5log以上、高純度の第Ⅸ因子製剤で7.4log、アルブミンで11.5log以上だった。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 1987年6月から1998年9月にかけて英国で出荷された血漿分画製剤の原料には、後にvCJDと診断された11人の供血者由来の血漿が含まれていたが、製剤に関連したvCJD症例は発生しておらず、製剤によってばらつきはあるものの製造工程にはプリオン除去効果があるとの報告である。 | 異常プリオンが本製剤の製造工程で効果的に除去されるとの実験成績を疫学的に裏付けた報告と言える。しかし、輸血によるvCJDに感染が示唆されていることから、今後も情報の収集に努める。尚、日本赤十字社はvCJD他の血液を介する感染防止の目的から、輸血歴のあるドナー、および英国を含む欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。特に英国については、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980年～1996年に1日以上の英国滞在歴のある方からの献血を制限している。 |

**使用上の注意記載状況・その他参考事項等**

抗HBs人免疫グロブリン「日赤」

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

# Epidemiology, Risk Assessment and Transmission

## P04.101

**Development of a Standardised Approach to Assess the Effectiveness of Current and New Decontamination Technologies against TSE Agents**

Hesp, IJR; Kirby, E; Dickinson, J; Dennis, M; Cornwall, M; Raven, NDH; Sutton, JM
*Health Protection Agency, Research, UK*

Background: The development of inactivation methods for Transmissible spongiform encephalopathies (TSEs) is an urgent requirement in relation to the potential for iatrogenic transmission of variant Creutzfeldt Jakob Disease (vCJD). The evaluation of the effectiveness of such methodologies requires a highly sensitive and specific assay or a combination of assays. With current cellular and biochemical based assays still in development, the bioassay remains the accepted approach to assess effectiveness; however, careful matching between the TSE strain and host species is required to help ensure that the risks are appropriately evaluated with regard to vCJD transmission.

Aims: The project aims to develop a robust system to assess proposed TSE inactivation technologies focusing on a model using the TSE strain, BSE-301V, designed to mimic the key features of possible vCJD transmission via contaminated *surgical instruments. The dynamic range of the model was determined using a titration* series of infectivity which in the first instance was 'tested' using a conventional autoclave based process.

Methods. BSE-301V infected mouse brain homogenate, previously titrated to $x10^9$ $ID_{50}$ per gram, was dried onto the surface of surgical steel suture wires using a standardised process. Wires were implanted i.c. into VM mice and monitored for clinical symptoms for up to 550 days.

Results: For the wire-based titration series clinical symptoms were observed in animals from groups across a 6-log dilution range, however, at dilutions below $10^{-3}$ transmission rates fell below 50%, suggesting that the useful range is around 4-logs. Data will be presented comparing the surface bound titration results with the equivalent in-solution titration series. The ongoing results from the decontamination studies will also be presented in relation to the titration data generated.

Conclusions: Methods have been established to ensure a consistent exposure of wires to the decontamination process with no further manipulations of the carriers post processing. Using this protocol a titration series has been established for BSE-301V on surgical steel that potentially covers a 4-log range. The use of these protocols to evaluate novel prion decontamination methods will be discussed.

## P04.102

**Has vCJD been Transmitted by Human Blood Plasma Products? 20 Years and Counting**

Foster, P
*Scottish National Blood Transfusion Service, Protein Fractionation Centre, UK*

The diagnosis of vCJD in a patient whose plasma had previously been used in the preparation of blood plasma products by the NHS led to the decision in 1998 that the preparation of plasma derivatives from UK-donor plasma should cease as a precautionary measure. Since then, plasma products have either been manufactured by the NHS, using plasma purchased from the USA and Europe, or purchased directly from commercial companies.

It is now known that donations from 11 individuals, later diagnosed with vCJD, had been included in the preparation of a total of 175 batches of different plasma products that were released for use between June 1987 and September 1998. No cases of vCJD have been associated with these products, although 20 years have elapsed since the first implicated batches were released for use. This contrasts with 3 instances of *probable transmission of vCJD by red cells in which symptoms of vCJD developed in* recipients 6.5 years, 7.8 years and 8.3 years after transfusion.

There are a number of possible explanations for the apparent absence of transmision by plasma products.

(1) Prion infectivity was not present in the donated plasma.
(2) Prion infectivity was present in the donated plasma but not in the manufactured products, due to dilution or removal of infectivity by the manufacturing process.
(3) Prion infectivity was present in manufactured product(s) but has not resulted in clinical symptoms of vCJD because of either a prolonged incubation period or a lack of suceptibility in recipients.
The methods used for the manufacture of blood plasma products by the Scottish National Blood Transfusion Service have been examined to determine the extent to which removal of prions might have occurred. These experiments indicate a possible overall prion reduction of 2.7 logs for intermediate-purity factor VIII concentrate (Z8), 3.0 logs for intermediate-purity factor IX concentrate (DEFIX), 5.8 logs for thrombin, ≥6.2 logs for fibrinogen, ≥6.5 logs for immunoglobulin, 7.4 logs for high-purity factor IX concentrate and ≥11.5 logs for albumin.

## P04.103

**Femtograms-Detection of PrPSc in Biological Samples using Chemically Synthesized RNA-Aptamer**

Nagata, T[1]; Yokoyama, T[2]; Sekiya, S[3]; Nishikawa, S[3]; Noda, K[1]
*[1]National Veterinary Assay Laboratory, Japan; [2]National Institute for Animal Health, Japan; [3]National Institute of Advanced Industrial Science and Technology (AIST), Japan*

For the safety of biological products, it is one of our major concerns to reduce the TSE-risk of cattle-blood derived materials such as serum and plasma. For the detection of possibly contaminated abnormal isoform of prion protein (PrPSc) in the biological samples, it is indispensable to develop a highly sensitive PrP detection procedure. Here, we have developed an aptamer-beads PrP-concentration procedure by using RNA-aptamer 60-3 which binds to recombinant mouse PrP with high affinity (Kd = 5.6 nM) (1).

The RNA-aptamer 60-3 was chemically synthesized employing a novel RNA synthetic method with a 2'-O-(2-cyanoethoxymethyl) protecting group (2), with 2'OMe-pyrimidine modification for RNase resistance, and conjugated with biotin. The aptamer was then bound to streptavidin-coated magnetic beads (60-3 aptamer-beads) and used for pull-down assays. The pulled-down PrPSc was analyzed by Western blotting.

The 60-3 aptamer-beads demonstrated the enrichment of PrPSc from the 20-million times diluted scrapie-infected mouse brain (50ml of 50ng brain equivalent /ml). Comparing to phosphotungstic acid (PTA) concentration method, the 60-3 aptamer-beads revealed more than 100 times efficiency in concentrating PrPSc spiked in bovine serum. Moreover, the 60-3 aptamer-beads showed binding ability to PrPSc in highly diluted BSE-infected bovine brain.

The present Aptamer-beads pull-down procedure enables us to perform a femtograms-detection of PrP. The procedure was also proven to be applicable to BSE-PrPSc. The present aptamer-beads system could serve as a resource for prion-removal column and serum prion assays, and potentially achieve the safety of the blood derived biological products.

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(1)S. Sekiya, K. Noda, F. Nishikawa, T. Yokoyama, P.K.R. Kumar and S. Nishikawa, J. Biochem. 139, 383-390, 2006.

(2)T. Ohgi, Y. Masutomi, K. Ishiyama, H. Kitagawa, Y. Shiba and J. Yano, Org. Lett. 7, 3477-3480, 2005.

## P04.104

**Survival of Prion Proteins in Environmental Matrices**

Maluquer de Motes, C[1]; Torres, JM[2]; Pumarola, M[3]; Girones, R[1]
*[1]University of Barcelona, Spain; [2]Centro de Investigación en Sanidad Animal, Spain; [3]Autonomous University of Barcelona, Spain*

Several publications have suggested the environment as a possible route of transmission, especially for sheep scrapie and cervid Chronic Wasting Disease (CWD). The role of the environment as a reservoir for these disorders is difficult to prove and faces a considerable lack of information. In this work, different methodologies have been developed to evaluate the survival and inactivation of TSE agents in environmental matrices.

Different slaughterhouse and urban sewage samples were spiked with diverse strains of either scrapie or BSE agents and kept under controlled conditions for extended periods of time. Aliquots of every experiment were sequentially collected and concentrated according to a methodology specifically selected for each type of matrix. Sensitivity of the methods developed was estimated among 2-10 µg of infected tissue. PrPres was finally detected by western blot. Films were then transformed into digital pictures, signal intensities were quantified and regression models were computed.

According to the results obtained, scrapie agent showed higher stability than BSE in all the environments studied. However, no significant differences were observed among mouse-passaged scrapie strains and sheep scrapie. The regression models provided t90 and t99 values (times of incubation necessaries for 90% and 99% reduction of PrPres levels). In urban sewage, i.e., t99 was estimated as about 50 and 22 days for scrapie and BSE respectively. In general, the effect of the matrix was clearly observed in all the experiments, showing up to a 6-8 fold higher reduction of PrPres levels in comparison to PBS controls.

As some of the inocula were titrated in terms of infectious doses, we approximated the decay of PrPres levels to the reduction of infectivity for both agents. In slaughterhouse wastewater, i.e., two-log reduction was observed for both agents after 30-35 days of incubation. Data on infectivity will be confirmed by a series of bioassay experiments.

別紙様式第2-1

No. 4

# 医薬品 研究報告 調査報告書

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| 販売名(企業名) | 赤血球濃厚液-LR「日赤」(日本赤十字社)<br>照射赤血球濃厚液-LR「日赤」(日本赤十字社) | | | 米国 |

**研究報告の概要**

○米国食品医薬品局(FDA)が血小板製剤中の細菌迅速検査用具を承認

米国食品医薬品局(FDA)は、輸血前血小板製剤の細菌汚染を検出する最初の迅速検査法を販売承認した。Platelet Pan Genera Detection (PGD) 検査システムは、病院の輸血現場において使用する使い捨ての検査機器である。当該検査法は製品出庫検査ではないが、自動採血の血小板に対する現行のQC検査法への追加が可能である。添付文書には、「製品出庫検査を検討しているユーザーは、適切な臨床試験を行うために、まず生物学的製剤評価研究センターと相談すべきである。全血由来血小板または白血球非除去血小板についての性能は不明であり、この検査のみによって、血小板の保存期間を延長すべきではない」と記載されている。

PGD検査システムがBacT/ALERTと実質的に同等であるという判断が2つの試験により支持され、培養検査後の補助的QC検査として使用できることが示された。この検査法は、サンプリングエラーにより早期の培養による細菌検出が不可能であったときでも、細菌汚染が検出可能であった。

汚染された血小板が輸血されるリスクを低減するため、血液センターでは供血から24時間後に培養検査を実施している。培養開始から24時間以内(供血から48時間以内)に判定を行い、汚染製剤は破棄される。しかし、サンプリングの限界から、培養時の細菌数が非常に少なく、検出されない場合がある。当該検査は医療機関向け製品であるが、一部センターで採用されている他の非培養法よりも優れているという意見が専門家の間で一致した。この検査法は標準的培養よりも感度が低いが、菌数が多くなる保管後期に検査が行われるため検出が容易である、とFDAは述べている。

PGD検査のプロトタイプは、輸血副作用に関連する多数の細菌種について試験された。開発企業の試験ではアフェレーシス血小板及び全血由来血小板7,889製剤から汚染された4製剤を検出した。これらは培養検査でも全例汚染が確認された。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 米国食品医薬品局は、輸血前血小板製剤の細菌汚染を検出するための最初の迅速検査、Platelet Pan Genera Detection検査システムを、医療機関における検査用として販売承認したとの報告である。 | 日本赤十字社では、輸血情報リーフレット等により、細菌感染やウイルス感染について医療機関へ情報提供し注意喚起している。また、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)における「本ガイドライン対象以外の病原体の取扱い イ. 細菌」に準じ細菌感染が疑われる場合の対応を医療機関に周知する。今後も細菌やウイルスの検出や不活化する方策について情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(18)

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# ABC NEWSLETTER

CURRENT EVENTS AND TRENDS IN BLOOD SERVICES

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**INSIDE:**



2007 #35 September 21, 2007

## FDA Approves Rapid Bacterial Detection Device for Platelets

The Food and Drug Administration has cleared for marketing the first rapid test to detect bacterial contamination in blood platelets prior to transfusion. The Platelet Pan Genera Detection (PGD) Test System, manufactured by Verax Biomedical Inc. of Worcester, Mass., is a disposable test strip device for use in a hospital transfusion setting. It is not a release test but can supplement current quality control testing methods for platelets collected with an automated instrument.

> **The package insert notes that "Users considering such release should first consult [the Center for Biologics Education and Research] for the appropriate clinical studies. [The performance of the PGD] to detect bacteria in whole blood-derived platelets or non-leukocyte reduced platelets is not known... [T]esting alone should not be used to extend the shelf life of platelets."**

Two studies supported the determination of substantial equivalence of the Platelet PGD Test system to BacT/ALERT testing and demonstrated the value of the PGD Test system as an adjunct QC test following culture testing, FDA said in a summary report. Testing at 72 hours using the PGD Test System was found to be substantially equivalent to testing by BacT/ALERT at 24 and 48 hours post collection. The PDG Test System was able to detect bacterial contamination when an early culture was unable to detect bacteria due to sampling errors.

> **"I am excited about the potential of this new test."**
>
> ***– Kevin Land, MD, Bonfils Blood Center***

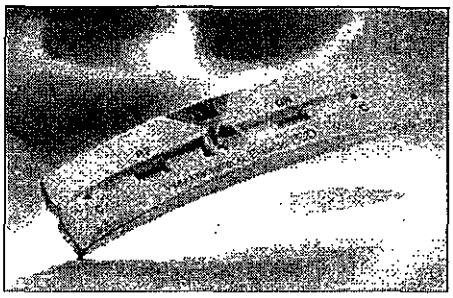
A 500uL platelet sample is insert in a sample well, and in about 20 minutes, a pink colored bar will appear in one of the two windows if either Gram-positive or Gram-negative bacteria are detected. Procedural controls at each end of the test cartridge change from yellow to blue violet when the appropriate volume of sample has been added to the cartridge and the test has run to completion.

"The clearance of a rapid test is a significant step in the detection of bacterial contamination of platelets for transfusion," said Jesse L. Goodman, MD, director of FDA's Center for Biologics Evaluation and Research.

(continued on page 2)

Rapid Platelet Test (continued from page 1)

Patients who are transfused with platelets contaminated with bacteria are at risk of developing a serious and potentially life-threatening infection of the blood stream. Bacterial contamination of platelets is the leading infectious cause of transfusion-related patient fatalities. The risk of a patient receiving a transfusion contaminated with bacteria is 1 in 5,000 – far greater than the risk of transmitting the hepatitis C virus (1 in 1.6 million) or HIV (1 in 1.9 million), the FDA said in a press release.



To reduce the risk of transfusing contaminated platelets, blood centers do culture-based testing of platelet samples 24 hours after the donation. The culture is read in the next 24 hours (within 48 hours of the donation), and contaminated units are discarded. However, the number of bacteria present at the time of culture may be so low that bacteria is not detected due to sampling limitations. Blood community professionals agreed that the test is more of a transfusion-end product but that it is better than other non-culture-based methods used by some blood centers and transfusion centers.

Kevin Land, MD, chief scientific and medical officer at Denver-based Bonfils Blood Center, told the *ABC Newsletter* that because the device is licensed to supplement current methodologies, "I don't see this test being widely implemented at this time due to the additional cost and time."

But, he added, "I am excited about the potential of this new test as it delays sampling of the product until it is being issued. The longer the delay prior to sampling, the more sensitive it should be to the presence of bacteria in the platelet component, even if the limit of detection is higher than current methodologies. It should definitely be an improvement over surrogate tests such as swirling and dipstick methods. Hopefully, the time between sampling and reading will decrease as the technology matures, as it likely represents a barrier to widespread use at this time."

Although the test system is less sensitive than standard cultures, testing is done later in storage when bacteria, if present, have multiplied, and thus are easier to detect, the FDA notes.

A prototype Platelet PGD test was tested against numerous bacterial species implicated in transfusion reactions. In an in-house study, Verax detected four contaminated platelet units in a mixed population of 7,889 apheresis and whole blood derived platelets. All four units were confirmed as contaminated by culture testing. (Sources: FDA press release, 9/18/07; Verax Web site) ♦

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**Publication Office**
America's Blood Centers
725 15th St NW, Suite 700, Washington, DC 20005
Tel: (202) 393-5725
E-mail: newsletter@americasblood.org

医薬品　研究報告　調査報告書

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|---|---|---|---|---|---|
| | | | 2008年1月15日 | 該当なし | |
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| 販売名（企業名） | 別紙のとおり | | | | |

**研究報告の概要**

問題点：2007年12月に中国で発生した鳥インフルエンザ感染患者は、感染患者から感染した可能性があることが明らかになった。しかし、ウイルスがヒトからヒトへ感染するタイプに変異した確証はない。

2008年1月10日、中国衛生当局は、2007年12月に江蘇省南京で発生した52歳男性の鳥インフルエンザ感染患者は、患者であった息子との濃厚な接触により感染したものであり、ウイルスの変異は認められていないことを明らかにした。

24歳の彼の息子は、2007年11月24日にH5N1型鳥インフルエンザに感染し、発熱、悪寒、その他の症状を発症した。同27日に下葉肺炎と診断され入院したが、2007年12月2日に死亡した。その後、男性の父親が、息子が死亡した翌日の12月3日に下葉肺炎と診断され入院した。H5N1型鳥インフルエンザウイルスに感染していることが判明したが、この父親は回復した。疫学調査により、この父親は息子との濃厚接触を通じて感染したことが分かった。

中国当局は、息子に感染したウイルスが家禽由来であり、変異がないことを確認していた。しかし、息子と父親、いずれにもヒトへの主な感染ルートである死亡した家禽との接触がないため、この息子がどのようにして感染したかは判明していない。地元当局は、2人の男性と密接な接触があった83人を厳重な監視下においていたが、これまで異常を示すものは1人もなかった。これで2003年以降の中国国内での鳥インフルエンザ感染事例は27例となり、うち17人が死亡している。

WHOは、鳥インフルエンザウイルスは、条件が整えば、感染力が増しヒト-ヒト感染を生じやすくなる可能性があると警告していた。このような変異は世界的大流行につながる。しかし、今回の事例では、ある患者からもう1人の患者へ疾患が伝播したことは明らかだが、ヒト-ヒト感染流行を生じる様な感染力は有していなかった。

使用上の注意記載状況・その他参考事項等

記載なし

| 報告企業の意見 | 今後の対応 |
|---|---|
| 別紙のとおり | 日本での流行の可能性を視野に入れ、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。 |

MedDRA10.1



19

| | |
|---|---|
| 一般的名称 | ①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ |
| 販売名(企業名) | ①献血アルブミン 20 “化血研”、②献血アルブミン 25 “化血研”、③人血清アルブミン “化血研” *、④ “化血研” ガンマーグロブリン、⑤献血静注グロブリン “化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500 注射用 |
| 報告企業の意見 | 鳥インフルエンザウイルスは、オルソミクソウイルス科のA型インフルエンザウイルス属に分類される。ウイルス粒子は 70～120nm の球形または多形性で、8本の分節状マイナス一本鎖 RNA を核酸として有する。エンベロープの表面に赤血球凝集素(HA)とノイラミダーゼ(NA)のスパイクを持ち、その抗原性により 16 種類の HA 亜型および 9 種類の NA 亜型に分類される。H5 亜型と H7 亜型の鳥インフルエンザウイルスの中には、家禽に高い致死率を示す高病原性のウイルスが存在する。現在、アジア中心に高病原性の H5N1 亜型による鳥インフルエンザが拡がっており、鳥からヒトだけでなく、ヒトからヒトへの感染事例も報告されている。<br>弊所の血漿分画製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。<br>各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第 1047 号、平成 11 年 8 月 30 日)」に従い、ウシウイルス性下痢ウイルス（BVDV)、仮性狂犬病ウイルス（PRV)、ブタパルボウイルス（PPV)、A型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告した鳥インフルエンザウイルスは、エンベロープの有無、核酸の種類等からモデルウイルスとしては BVDV が該当すると考えられるが、上記バリデーションの結果から、BVDV の除去・不活化効果を有することを確認している。<br>また、これまでに当該製剤による鳥インフルエンザウイルス感染の報告例は無い。<br>以上の点から、当該製剤は鳥インフルエンザウイルスに対する安全性を確保していると考える。 |

*現在製造を行っていない

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HEALTH

# China's latest human case of bird flu infected through close contact with ill son

www.chinaview.cn 2008-01-10 12:05:53 Print

BEIJING, Jan. 10 (Xinhua) -- Health authorities confirmed here on Thursday that the latest human case of bird flu in the eastern province of Jiangsu, which involved a 52-year-old father, came from close contact with his infected son and not a viral mutation.

The World Health Organization has warned that the virus that causes the illness -- if given sufficient opportunity -- would mutate into a form that is highly infectious and easily transmissible from person to person. Such a change could start a global outbreak.

However, this case -- although it involved the disease apparently passing from one person to another -- does not exactly fit the profile of an infectious human-to-human outbreak, and it has remained something of a puzzle.

"It has no biological features for human-to-human transmission," said Mao Qun'an, Health Ministry spokesman. An epidemiological investigation showed the father was infected through close contact with his son, he said.

The cases took place in the provincial capital, Nanjing. The son, 24, and the first to be infected, died on Dec. 2. The father was later confirmed to be infected with the H5N1 virus, which causes bird flu.

At the time, the ministry said experts had found that the virus that infected the son had originated with poultry and had not mutated. But it remained unclear how the son was infected in the first place, as neither man had any known contact with dead poultry -- the primary known source of the ailment for humans.

The young man, surnamed Lu, developed fever, chills and other symptoms on Nov. 24 and was hospitalized on Nov. 27 after being diagnosed with lower left lobe pneumonia. His father developed a fever and was hospitalized for lower lobe pneumonia on Dec. 3, the day after his son's death.

"The father has recovered," Mao said, adding that the cases have been effectively contained.

Local authorities had kept 83 people who had close contact with either man under close observation but none had shown unusual symptoms so far, according to the ministry.

The case of the Lu family, although unusual, is not the only one of its kind. Reuters reported last month that a similar case occurred in Pakistan.

The latest cases bring the number of confirmed human infections of bird flu in China to 27 since 2003, with 17 deaths.

A human-use bird flu vaccine has been in the second phase of clinical tests in Beijing by the Beijing-based vaccine producer Sinovac Biotech and the Chinese Center for Disease Control and Prevention.

Next

Editor: An Lu

HEALTH

# China's latest human case of bird flu infected through close contact with ill son

www.chinaview.cn 2008-01-10 12:05:53



It has proved "safe" and "effective" in the test, said Sinovac Biotech in late last month.

The major index of the vaccine all reached international standard and performed well in human body. None of the test takers were found with serious negative reaction, which proved that the vaccine was safe, it said.

Bird flu, or Avian influenza, is a contagious disease of animal origin caused by viruses that normally infect only birds and, less commonly, pigs.

China's Ministry of Agriculture said in early December that the possibilities of regional bird flu outbreaks were "very high" in the winter and coming spring.

Xinjiang in northwest China has reported an outbreak of bird flu since late December, leading to the death of more than 35,000 poultry.

The local government said the situation has been under control and no human infection has been found yet.

Previous

Editor: An Lu

# B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料３において集積報告を行っているため、添付していない）。

# 感染症定期報告の報告状況(2007/12/1～2008/2/29)

| 血対ID | 受理日 | 報告者名 | 一般名 | 生物由来成分名 | 原材料名 | 原産国 | 含有区分 | 文献 | 症例 | 適正使用措置 |
|---|---|---|---|---|---|---|---|---|---|---|
| 70166 | 2007/12/17 | 化学及血清療法研究所 | 乾燥スルホ化人免疫グロブリン | スルホ化人免疫グロブリンG | ヒト血液 | 米国、日本 | 有効成分 | 有 | 無 | 無 |
| 70167 | 2007/12/17 | 化学及血清療法研究所 | 乾燥濃縮人アンチトロンビンIII | アンチトロンビンIII | ヒト血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70168 | 2007/12/20 | 日本赤十字社 | 抗HBs人免疫グロブリン | 抗HBs人免疫グロブリン | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70169 | 2007/12/20 | 日本赤十字社 | 人赤血球濃厚液 | 人赤血球濃厚液 | 人血液 | 日本 | 有効成分 | 有 | 有 | 有 |
| 70170 | 2007/12/20 | 日本赤十字社 | 人全血液 | 人全血液 | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70171 | 2007/12/25 | ベネシス | ポリエチレングリコール処理抗破傷風人免疫グロブリン<br>乾燥抗破傷風人免疫グロブリン | 破傷風抗毒素 | 人血液 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70172 | 2007/12/28 | バクスター | 乾燥イオン交換樹脂処理人免疫グロブリン | 人免疫グロブリンG | 人血漿 | 米国 | 有効成分 | 無 | 無 | 無 |
| 70173 | 2007/12/28 | バクスター | 乾燥イオン交換樹脂処理人免疫グロブリン | 人血清アルブミン | 人血漿 | 米国 | 添加物 | 無 | 無 | 無 |
| 80001 | 2008/01/11 | 富士フイルムRIファーマ | テクネチウム大凝集人血清アルブミン(99mTc) | テクネチウム大凝集人血清アルブミン(99mTc) | ヒト血液 | 米国 | 有効成分 | 有 | 無 | 無 |
| 80002 | 2008/01/23 | ベネシス | 人ハプトグロビン | 人ハプトグロビン | 人血液 | 非献血:米国、献血:日 | 有効成分 | 有 | 無 | 無 |
| 80003 | 2008/01/25 | ノボノルディスクファーマ | エプタコグ アルファ(活性型)(遺伝子組換え) | ウシ新生仔血清 | ウシ血液 | ニュージーランド | 製造工程 | 無 | 無 | 無 |
| 80004 | 2008/01/25 | ノボノルディスクファーマ | エプタコグ アルファ(活性型)(遺伝子組換え) | ブタ膵臓由来トリプシン | ブタ膵臓(抽出物) | 不明 | 製造工程 | 無 | 無 | 無 |
| 80005 | 2008/01/25 | ノボノルディスクファーマ | エプタコグ アルファ(活性型)(遺伝子組換え) | ウシ胎仔血清 | ウシ血液 | ニュージーランド、オーストラリア、米国及びカナダ | 製造工程 | 無 | 無 | 無 |
| 80006 | 2008/01/25 | ノボノルディスクファーマ | エプタコグ アルファ(活性型)(遺伝子組換え) | エプタコグ アルファ(活性型)(遺伝子組換え) | エプタコグ アルファ(活性型)(遺伝子組換え) | 該当しない | 有効成分 | 無 | 無 | 無 |
| 80007 | 2008/01/25 | CSLベーリング | 乾燥濃縮人アンチトロンビンIII | 乾燥濃縮人アンチトロンビンIII | ヒト血液 | 米国、ドイツ、オーストリア | 有効成分 | 有 | 無 | 無 |
| 80008 | 2008/01/25 | 日本製薬 | 乾燥人血液凝固第IX因子複合体 | 血液凝固第IX因子複合体 | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 80009 | 2008/01/28 | 日本メジフィジックス | 放射性医薬品基準ガラクトシル人血清アルブミンジエチレントリアミン五酢酸テクネチウム(99mTc)注射液 | ガラクトシル人血清アルブミンジエチレントリアミン五酢酸テクネチウム(99mTc) | 生物学的製剤基準人血清アルブミン | 日本 | 有効成分 | 無 | 無 | 無 |
| 80010 | 2008/01/29 | 化学及血清療法研究所 | 乾燥濃縮人血液凝固第VIII因子 | 血液凝固第VIII因子 | ヒト血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 80011 | 2008/01/30 | バクスター | 加熱人血漿たん白 | 人血清アルブミン | 人血漿 | 米国 | 有効成分 | 無 | 有 | 無 |
| 80012 | 2008/01/30 | バクスター | ルリオクトコグ アルファ(遺伝子組換え) | ルリオクトコグ アルファ(遺伝子組換え) | 遺伝子組換えチャイニーズハムスター卵巣細胞株 | 該当なし | 有効成分 | 無 | 無 | 無 |
| 80013 | 2008/01/30 | バクスター | ルリオクトコグ アルファ(遺伝子組換え) | アプロチニン | ウシ肺 | ニュージーランド | 製造工程 | 無 | 無 | 無 |
| 80014 | 2008/01/30 | バクスター | ルリオクトコグ アルファ(遺伝子組換え) | インスリン(抗第VIII因子モノクローナル抗体製造用) | ウシ膵臓 | 米国 | 製造工程 | 無 | 無 | 無 |
| 80015 | 2008/01/30 | バクスター | ルリオクトコグ アルファ(遺伝子組換 | ウシ血清アルブミン | ウシ血液 | 米国 | 製造工程 | 無 | 無 | 無 |
| 80016 | 2008/01/30 | バクスター | ルリオクトコグ アルファ(遺伝子組換え) | ウシ胎児血清(抗第VIII因子モノクローナル抗体製造用) | ウシ血液 | オーストラリア | 製造工程 | 無 | 無 | 無 |
| 80017 | 2008/01/30 | バクスター | ルリオクトコグ アルファ(遺伝子組換 | 人血清アルブミン | 人血漿 | 米国 | 添加物 | 無 | 無 | 無 |
| 80018 | 2008/01/30 | バクスター | ルリオクトコグ アルファ(遺伝子組換え) | 培養補助剤(抗第VIII因子モノクローナル抗体製造用-1) | ウシ血液 | 米国 | 製造工程 | 無 | 無 | 無 |
| 80019 | 2008/01/30 | バクスター | ルリオクトコグ アルファ(遺伝子組換え) | 培養補助剤(抗第VIII因子モノクローナル抗体製造用-2) | ウシ肝臓 | 米国又はカナダ | 製造工程 | 無 | 無 | 無 |

| 血対ID | 受理日 | 報告者名 | 一般名 | 生物由来成分名 | 原材料名 | 原産国 | 含有区分 | 文献 | 症例 | 適正使用措置 |
|---|---|---|---|---|---|---|---|---|---|---|
| 80020 | 2008/02/15 | ベネシス | 人血清アルブミン<br>乾燥濃縮人アンチトロンビンIII<br>人ハプトグロビン<br>乾燥濃縮人血液凝固第VIII因子 | ヘパリン | ブタ小腸粘膜 | 中国 | 製造工程 | 無 | 無 | 無 |
| 80021 | 2008/02/15 | 富士フイルムRIファー | ヨウ化人血清アルブミン(131I) | ヨウ化人血清アルブミン(131I) | ヒト血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 80022 | 2008/02/22 | CSLベーリング | 人C1-インアクチベーター | 人C1-インアクチベーター | ヒト血液 | 米国、ドイツ、オーストリア | 有効成分 | 有 | 無 | 無 |
| 80023 | 2008/02/22 | CSLベーリング | 人血清アルブミン<br>人免疫グロブリンG<br>破傷風抗毒素<br>フィブリノゲン加第XIII因子<br>ペプシン処理人免疫グロブリンG<br>乾燥濃縮人アンチトロンビンIII | ヘパリンナトリウム | ブタ腸粘膜 | 中国 | 製造工程 | 無 | 無 | 無 |
| 80024 | 2008/02/22 | CSLベーリング | 乾燥pH4処理人免疫グロブリン | ペプシン | ブタ胃粘膜 | 米国 | 製造工程 | 有 | 無 | 無 |
| 80025 | 2008/02/25 | 日本製薬 | ①加熱人血漿たん白<br>②人血清アルブミン(5%)<br>③人血清アルブミン(20%)<br>④人血清アルブミン(25%)<br>⑤乾燥ポリエチレングリコール処理人免疫グロブリン<br>⑥トロンビン<br>⑦乾燥濃縮人アンチトロンビンIII<br>⑧人免疫グロブリン<br>⑨乾燥人血液凝固第IX因子複合体 | ヘパリン | ブタ腸粘膜 | ブラジル | ①-⑧製造工程<br>⑨添加物・製造工程 | 無 | 無 | 無 |
| 80026 | 2008/02/27 | 化学及血清療法研究所 | 乾燥抗破傷風人免疫グロブリン | 抗破傷風人免疫グロブリン | ヒト血液 | 米国 | 有効成分 | 有 | 無 | 無 |
| 80027 | 2008/02/27 | 化学及血清療法研究所 | 乾燥人血液凝固第IX因子複合体<br>乾燥濃縮人血液凝固第IX因子 | 血液凝固第IX因子 | ヒト血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 80028 | 2008/02/27 | 化学及血清療法研究所 | 乾燥人血液凝固第IX因子複合体<br>乾燥濃縮人血液凝固第IX因子<br>乾燥濃縮人アンチトロンビンIII<br>人免疫グロブリン<br>フィブリノゲン加第XIII因子<br>乾燥濃縮人活性化プロテインC<br>ヒスタミン加人免疫グロブリン<br>トロンビン<br>乾燥スルホ化人免疫グロブリン<br>人血清アルブミン<br>乾燥ペプシン処理人免疫グロブリン | ヘパリンナトリウム | ブタ腸粘膜 | 中国、フランス、米国、カナダ | 製造工程 | 無 | 無 | 無 |
| 80029 | 2008/02/28 | バクスター | ルリオクトコグ アルファ(遺伝子組換え) | ルリオクトコグ アルファ(遺伝子組換え) | 遺伝子組換えチャイニーズハムスター卵巣細胞株 | 該当なし | 有効成分 | 無 | 無 | 無 |

別紙様式第4

# 感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第9回 | 1 | 感染症および寄生虫症 | 細菌感染 | 日本 | 男 | 63 | 2007/10/5 | ②軽快 | 症例報告 | 当該製品 | 2007/10/19提出、識別番号1-07000105 未完了報告 |
| | 2 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 76 | 2007/9/20 | ⑥不明 | 症例報告 | 当該製品 | 2007/10/18提出、識別番号1-07000103 未完了報告 |
| | 3 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 81 | 2007/9/12 | ③未回復 | 症例報告 | 当該製品 | 2007/10/15提出、識別番号1-07000101 未完了報告 |
| | 4 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 70 | 2007/9/3 | ⑤死亡 | 症例報告 | 当該製品 | 2007/9/28提出、識別番号1-07000095 未完了報告 |
| | 5 | 感染症および寄生虫症 | 敗血症性ショック | 日本 | 女 | 58 | 2007/9/2 | ②軽快 | 症例報告 | 当該製品 | 2007/9/18提出、識別番号1-07000090 未完了報告 |
| | 6 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 67 | 2007/8/30 | ③未回復 | 症例報告 | 当該製品 | 2007/10/9提出、識別番号1-07000097 未完了報告 |
| | 7 | 感染症および寄生虫症 | 細菌感染 | 日本 | 男 | 71 | 2007/8/29 | ②軽快 | 症例報告 | 当該製品 | 2007/9/20提出、識別番号1-07000091 未完了報告 |
| | 8 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 53 | 2007/8/28 | ②軽快 | 症例報告 | 当該製品 | 2007/10/19提出、識別番号1-07000104 未完了報告 |
| | 9 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 68 | 2007/8/17 | ③未回復 | 症例報告 | 当該製品 | 2007/9/11提出、識別番号1-07000087 未完了報告 |
| | 10 | 感染症および寄生虫症 | 細菌感染 | 日本 | 男 | 93 | 2007/8/10 | ①回復 | 症例報告 | 当該製品 | 2007/8/28提出、識別番号1-07000081 未完了報告 |
| | 11 | 感染症および寄生虫症 | サイトメガロウイルス感染 | 日本 | 女 | 1 | 2007/8/2 | ③未回復 | 症例報告 | 当該製品 | 2007/8/16提出、識別番号1-07000079 未完了報告 |
| | 12 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 64 | 2007/7/27 | ③未回復 | 症例報告 | 当該製品 | 2007/8/28提出、識別番号1-07000082 未完了報告 |
| | 13 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 63 | 2007/7/26 | ②軽快 | 症例報告 | 当該製品 | 2007/8/16提出、識別番号1-07000076 未完了報告 |
| | 14 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 66 | 2007/7/26 | ③未回復 | 症例報告 | 当該製品 | 2007/9/3提出、識別番号1-07000085 未完了報告 |
| | 15 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 67 | 2007/7/12 | ⑥不明 | 症例報告 | 当該製品 | 2007/7/26提出、識別番号1-07000067 未完了報告 |
| | 16 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 67 | 2007/7/12 | ③未回復 | 症例報告 | 当該製品 | 2007/8/22提出、識別番号1-07000067 未完了報告（15番と同一症例） |
| | 17 | 感染症および寄生虫症 | 細菌感染 | 日本 | 女 | 64 | 2007/7/12 | ②軽快 | 症例報告 | 当該製品 | 2007/7/26提出、識別番号1-07000068 未完了報告 |
| | 18 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 52 | 2007/7/9 | ③未回復 | 症例報告 | 当該製品 | 2007/8/16提出、識別番号1-07000078 未完了報告 |
| | 19 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 62 | 2007/7/6 | ⑥不明 | 症例報告 | 当該製品 | 2007/7/25提出、識別番号1-07000064 未完了報告 |
| | 20 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 82 | 2007/6/21 | ③未回復 | 症例報告 | 当該製品 | 2007/9/13提出、識別番号1-07000089 未完了報告 |
| | 21 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 66 | 2007/6/20 | ③未回復 | 症例報告 | 当該製品 | 2007/7/20提出、識別番号1-07000061 未完了報告 |
| | 22 | 感染症および寄生虫症 | 敗血症性ショック | 日本 | 女 | 84 | 2007/6/19 | ③未回復 | 症例報告 | 当該製品 | 2007/7/4提出、識別番号1-07000052 未完了報告 |
| | 23 | 感染症および寄生虫症 | 細菌感染 | 日本 | 男 | 81 | 2007/6/16 | ①回復 | 症例報告 | 当該製品 | 2007/7/25提出、識別番号1-07000065 未完了報告 |
| | 24 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 64 | 2007/6/13 | ③未回復 | 症例報告 | 当該製品 | 2007/7/5提出、識別番号1-07000055 未完了報告 |
| | 25 | 感染症および寄生虫症 | 伝染性紅斑 | 日本 | 男 | 71 | 2007/6/12 | ①回復 | 症例報告 | 当該製品 | 2007/7/11提出、識別番号1-07000057 未完了報告 |
| | 26 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 17 | 2007/6/6 | ③未回復 | 症例報告 | 当該製品 | 2007/7/4提出、識別番号1-07000051 未完了報告 |
| | 27 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 81 | 2007/6/4 | ①回復 | 症例報告 | 当該製品 | 2007/10/1提出、識別番号1-07000096 未完了報告 |
| | 28 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 79 | 2007/6/1 | ②軽快 | 症例報告 | 当該製品 | 2007/6/28提出、識別番号1-07000047 未完了報告 |
| | 29 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 79 | 2007/6/1 | ②軽快 | 症例報告 | 当該製品 | 2007/7/4提出、識別番号1-07000047 未完了報告（28番と同一症例） |
| | 30 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 84 | 2007/6/1 | ③未回復 | 症例報告 | 当該製品 | 2007/7/5提出、識別番号1-07000053 未完了報告 |
| | 31 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 75 | 2007/5/29 | ⑥不明 | 症例報告 | 当該製品 | 2007/6/25提出、識別番号1-07000044 未完了報告 |
| | 32 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 75 | 2007/5/29 | ⑥不明 | 症例報告 | 当該製品 | 2007/8/9提出、識別番号1-07000044 未完了報告（31番と同一症例） |
| | 33 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 75 | 2007/5/29 | ③未回復 | 症例報告 | 当該製品 | 2007/8/22提出、識別番号1-07000044 未完了報告（31番、32番と同一症例） |
| | 34 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 38 | 2007/5/29 | ②軽快 | 症例報告 | 当該製品 | 2007/7/13提出、識別番号1-07000058 未完了報告 |



MedDRA/J Ver. 10.0J

別紙4

別紙様式第4

## 感染症発生症例一覧

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 64 | 2007/5/28 | ⑥不明 | 症例報告 | 当該製品 | 2007/6/25提出、識別番号1-07000045　未完了報告 |
| 36 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 64 | 2007/5/28 | ⑥不明 | 症例報告 | 当該製品 | 2007/8/9提出、識別番号1-07000045　取り下げ（35番と同一症例） |
| 37 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 83 | 2007/5/23 | ⑥不明 | 症例報告 | 当該製品 | 2007/7/13提出、識別番号1-07000060　未完了報告 |
| 38 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 77 | 2007/5/17 | ⑥不明 | 症例報告 | 当該製品 | 2007/6/14提出、識別番号1-07000037　未完了報告 |
| 39 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 73 | 2007/5/16 | ⑥不明 | 症例報告 | 当該製品 | 2007/5/30提出、識別番号1-07000029　未完了報告 |
| 40 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 51 | 2007/5/1 | ⑥不明 | 症例報告 | 当該製品 | 2007/5/25提出、識別番号1-07000026　未完了報告 |
| 41 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 51 | 2007/5/1 | ③未回復 | 症例報告 | 当該製品 | 2007/6/8提出、識別番号1-07000026　未完了報告（40番と同一症例） |
| 42 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 82 | 2007/5/1 | ⑤死亡 | 症例報告 | 当該製品 | 2007/6/14提出、識別番号1-07000038　未完了報告 |
| 43 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 42 | 2007/4/27 | ③未回復 | 症例報告 | 当該製品 | 2007/5/21提出、識別番号1-07000023　未完了報告 |
| 44 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 42 | 2007/4/27 | ②軽快 | 症例報告 | 当該製品 | 2007/8/7提出、識別番号1-07000023　取り下げ（43番と同一症例） |
| 45 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 73 | 2007/4/25 | ③未回復 | 症例報告 | 当該製品 | 2007/6/6提出、識別番号1-07000032　未完了報告 |
| 46 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 73 | 2007/4/25 | ⑥不明 | 症例報告 | 当該製品 | 2007/8/7提出、識別番号1-07000032　取り下げ（45番と同一症例） |

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| 70169 | 2007/12/20 | 日本赤十字社 | 人赤血球濃厚液 | 人赤血球濃厚液 |

別紙様式第４

# 感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期（年/月/日） | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第9回 | 9-1 | 感染症および寄生虫症 | Ｃ型肝炎 | 日本 | 女 | 40歳代 | 不明 | 不明 | 症例報告 | 当該製品 | 識別番号：07000164<br>報告日：2007年12月28日<br>MedDRA: Version(10.1) |
| | 9-2 | 感染症および寄生虫症 | Ｂ型肝炎 | 日本 | 女 | 26歳 | 2007/11/5 | 未回復 | 症例報告 | 当該製品 | 識別番号：07000143<br>報告日：2007年12月13日<br>MedDRA: Version(10.1) |
| 第8回 | 該当なし | | | | | | | | | | |
| 第7回 | 該当なし | | | | | | | | | | |
| 第6回 | 該当なし | | | | | | | | | | |
| 第5回 | 該当なし | | | | | | | | | | |
| 第4回 | 1-2 | 臨床検査 | Ｃ型肝炎抗体陽性 | 日本 | 男 | 76歳 | 2003/9/19 | 不明 | 症例報告 | 当該製品 | 登録番号：A03-120<br>報告日：2005年4月28日<br>第1回症例番号1-2において報告したもの（未完了報告）の取り下げ報告<br>MedDRA: Version(7.1) |
| 第3回 | 該当なし | | | | | | | | | | |

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別紙様式第４

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期（年/月/日） | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第２回 | 該当なし | | | | | | | | | | |
| 第１回 | 1-1 | 臨床検査 | Ｂ型肝炎表面抗原陽性 | 日本 | 男性 | 72歳 | 2003/7/18 | 不明 | 症例報告 | 当該製品 | 識別番号：Ａ03-40<br>報告日：2003年９月５日<br>MedDRA: Version(7.1) |
| | 1-2 | 臨床検査 | Ｃ型肝炎抗体陽性 | 日本 | 男性 | 76歳 | 2003/9/19 | 不明 | 症例報告 | 当該製品 | 登録番号：Ａ03-120<br>（未完了報告<br>報告日：2003年10月３日）<br>MedDRA: Version(7.1) |

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| 80011 | 2008/01/30 | バクスター | 加熱人血漿たん白 | 人血清アルブミン |